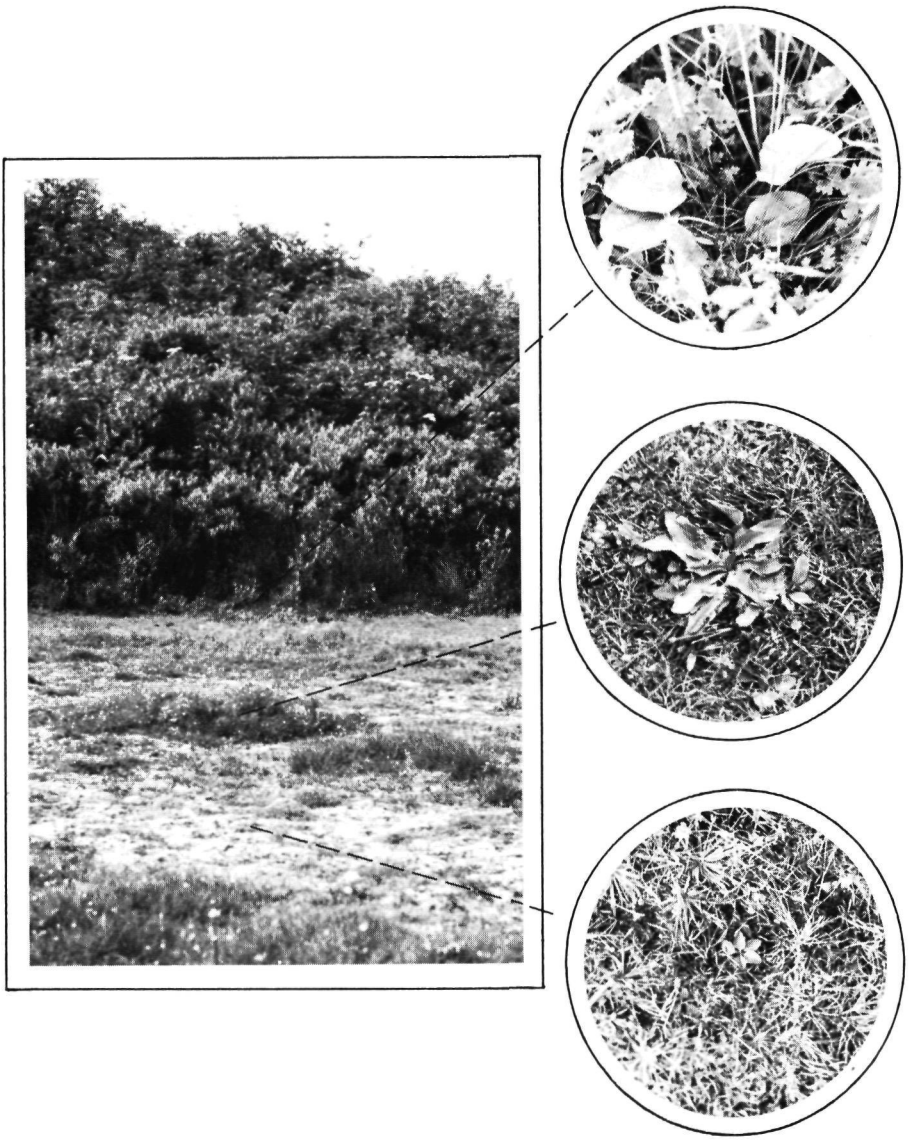
VARIATION IN LIFE-HISTORY CHARACTERISTICS BETWEEN AND WITHIN POPULATIONS OF PLANTAGO MAJOR L.

L.A.P. LOTZ



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VARIATION IN LIFE-HISTORY CHARACTERISTICS BETWEEN AND WITHIN POPULATIONS OF PLANTAGO MAJOR L.

een wetenschappelijke proeve op het gebied van de Natuurwetenschappen

Proefschrift

ter verkrijging van de graad van doctor aan de Katholieke Universiteit te Nijmegen, volgens besluit van het college van decanen in het openbaar te verdedigen op vrijdag 8 december 1989 des namiddags te 1.30 uur precies

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Voor Vader en Moeder,
voor Judica

Omslag illustratie: W. Verholt

Legend to cover photo: Environmental variation at the Oostvoornse Meer site with in close-up *Plantago major* in, from top to bottom, the *Hippophae* shrubs, the small elevations and the low-lying area (Chapter 5 - 8).

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VOORWOORD

Dit proefschrift geeft een weerspiegeling van verkregen populatiebiologische kennis en onderzoekservaring. Daartoe waren drie verschillende "omgevingen" met name van belang. In de Nederlandse Jeugdbond voor Natuurstudie kreeg ik de gelegenheid me te verwonderen over de rijkdom aan variatie in tal van aspecten van biologische systemen en zette daar de eerste schreden om, samen met anderen, deze verwondering door middel van onderzoek aan te vullen met kennis en inzicht. Tijdens mijn studie aan de Rijksuniversiteit te Groningen werd de akademische basis gelegd voor dit proefschrift. Prof. Dr. ir. P.J.C. Kuiper, als man op de achtergrond, leerde mij op stimulerende wijze diverse relevante onderzoeksgebieden te verkennen. De derde omgeving is het Instituut voor Oecologisch Onderzoek, waar het betreffende populatiebiologisch onderzoek aan de grote weegbree werd uitgevoerd. Directeur Dr. J.W. Woldendorp, en de achtereenvolgende Hoofden van het Biologisch Station "Weevers' Duin" Prof. Dr. C.W.P.M. Blom en Prof. Dr. J.M.M. van Damme, zorgden voor een zeer stimulerend en kritisch onderzoeksmilieu.

Velen hebben aandeel gehad in het verzamelen van de verschillende onderzoeksresultaten. Ten eerste, de Weevers'Duiners, werkelijk geen uitgezonderd, toonden grote bereidheid om op bepaalde momenten een helpende hand te bieden. Sep Troelstra en medewerkers ben ik zeer erkentelijk voor de bodemkundige inbreng. De studenten Hugo Coops, Han Olff, Leo Spoormakers en Karin Verspui bewerkten een doctoraalvak binnen het onderzoek en leverden daarmee een substantiele bijdrage, evenals de stagiaires die meehielpen met het onderzoek in het kader van hun studie aan verschillende analistenopleidingen. Verder waren vele NJN-ers, en andere Voornegangers bereid te helpen met het verrichten van veldwerk en het oogsten van planten uit kasproeven. Erna Vlot hielp met het typen van tabellen en Wim Verholt en Nicole Tolmeijer verzorgden het grafisch werk. Manuscripten werden gelezen en van kritisch kommentaar voorzien door Frank Berendse, Wilke van Delden, Jan van Groenendael, Piet Kuiper, Hans Lambers, Han Olff, Peter van Tienderen, Onno van Tongeren en Jan Woldendorp. Allen wil ik hartelijk danken.

Tenslotte, jij, Judica. Bedankt voor heel veel .. !

Bij elke verovering op 't gebied van het onbekende, roept ons de Natuur duidelyk toe: *what next?* 't Zou een leugenaar zyn die, iets gevonden hebbende, zich beroemde: we zyn er! Integendeel, elke oplossing is de moeder van nieuwe vraagstukken.

Multatuli, uit Idee 869

Ideeën, derde bundel (1868-1871)
In: Volledig Werk, 4 (1973),
G.A. Van Oorschot, Amsterdam.

CONTENTS

CHAPTER 1
GENERAL INTRODUCTION

Genetic differentiation and phenotypic plasticity in life-history characteristics

In modern plant population ecology a great deal of attention has been focused on the variation in and evolution of life-history characteristics (Dirzo and Sarukhan 1984, Haeck and Woldendorp 1985). Life-history characteristics are directly involved in survival and reproduction of organisms: e.g. size at birth, age and size at reproduction, age- and size-specific birth and death rates, number of offspring (Cole 1954). Insight into the factors that cause variation in life-history characteristics is essential in order to be able to understand the dynamics of size and the composition of populations, in relation to the environment and is therefore, invaluable in many areas of fundamental as well as applied ecology.

In the analysis of the variation in life-history characteristics two features can be distinguished: genetic differentiation as a result of different selection regimes and phenotypic plasticity. *Phenotypic plasticity* is defined by Bradshaw (1965) as the amount by which an individual genotype can be modified by its environment. He reviewed the results of many studies, and showed that phenotypic plasticity is genetically controlled and specific to the characteristic of the organism and its relation to particular environmental influences.

Bradshaw (1965), Blom and Lotz (1985), Via and Lande (1985) and Schlichting (1986) stated that a plastic response of a plant may be adaptive in an environment that is heterogeneous in space or time. Adaptive plastic responses might develop if plants experience different environments, and if in each of those environments different life histories are "optimal", while appropriate genetic variation is present.

Many authors have tried to assess the relative importance of genetic differentiation and phenotypic plasticity in determining variation in life-history traits within a species (e.g. Bradshaw 1972, Scheiner and Goodnight 1984, Schlichting 1986, Wolff 1988, and references cited in these papers). Bradshaw (1972) reviewed many studies on genetic differentiation in plants, e.g. due to small-scale spatial differences in metal contamination of the soil. He stated that in heterogeneous environments the distribution of a species merely depends on its capacity to evolve locally, genetically distinct

populations, and that the relative importance of plasticity might be small, even for small-scale spatial variation in environment, as genetic differentiation between populations can occur easily. In contrast to this, several authors suggested that phenotypic plasticity played an important role in enabling an individual plant to grow and survive in a wide range of environments (e.g. Caswell 1983, Fenner 1987, Heathcote et al. 1987). For example, Heathcote et al. (1987) suggested that phenotypic plasticity in the production of adventious roots in relation to a flooding regime is important to the wide ecological amplitude of *Carex flacca*. Schaal (1984), Scheiner and Goodnight (1984) and Wolff (1988) stressed the possibility that a combination of both genetic differentiation and phenotypic plasticity may be relevant to the distribution of species and the evolution within populations. However, generalisations with respect to the relative importance of genetic differentiation and phenotypic plasticity and fitness of plants might be difficult to formulate because of:

1. the aforementioned specificness of phenotypic plasticity both in the characteristic itself as well as in relation to particular environmental influences
2. the fact that the environment can vary both in space and time in a complex way so that the relationship between environmental variability and observed variation in life-history characteristics is difficult to detect (e.g. Lacey et al. 1983)
3. the complexity of life-history functions; characteristics related to either survival or reproduction may often interact (Law et al. 1977, Reznick 1985)
4. the dependence of the relative importance of genetic differentiation and phenotypic plasticity on species characteristics, e.g. sex systems and other characteristics related to potentials of gene flow (Van Damme 1983, Levin 1984, Lloyd 1984, Loveless and Hamrick 1984)
5. the influences of processes in the past, e.g. the foundation of population and previous selection regimes, both of which may have influenced the level of genetic variation (Bradshaw 1972, Carson and Templeton 1984, Levin 1984).

Therefore, any study on the relationship between variation in life-history characteristics and environmental variability should ideally incorporate data with respect to these five items.

Constraints on the variablity in life-history characteristics

Studying those factors that constrain variation in life-history characteristics is essential to gain new insight into the adaptation of plants

to their environment. In this respect many open questions are still left in *trade-off* relationships between life-history characteristics (e.g. Reznick 1985, Werner 1985, Schlichting 1986). One may consider trade-offs with reference to:

1. internal compensating mechanisms which result in negatively correlated characteristics in a single phenotype (physiological point of view), 2. patterns of morphogenesis, e.g. architectural constraints or patterns of translocation due to age or life stage (Watson and Casper 1984), and 3. an evolutionary context.

Trade-offs in the evolutionary context are based on the fact that characteristics related to either survival or reproduction interact negatively. In other words, an improvement in some characteristic related to fitness is associated with a decline in some other fitness-related characteristic (Reznick 1985). One of the main problems in the analysis of this type of trade-offs is the fact that fitness of individual genotypes is generally difficult to determine, e.g. due to the ability to produce seeds over a long period (Endler 1986). In addition, evaluating trade-offs in an evolutionary context requires demonstrating a genetic basis for the negative relationship (Reznick 1985).

Contents of this thesis

The principal aim of the study presented in this thesis is to relate variation in life-history characteristics to environmental variability within and between populations of a grassland species. *Plantago major* L. generally has a perennial life history with iteroparitive reproduction. However, in some environments with a high temporal variability in growing conditions, e.g. river banks, plants have an annual life history. For this reason, and because *P. major* occurs in contrasting habitats (e.g. with respect to structure and nutrient content of the soil and to level of interspecific competition) this species seems a suitable object for study. The life stages and life-history characteristics, investigated in this study, are summarized in Table 1.

The following specific questions will be addressed:

1. What is the amount of variation in life-history characteristics within and between populations of *P. major*?
2. What is the relative importance of genetic differentiation and phenotypic plasticity in this variation?
3. With respect to what life-history characteristics may natural selection have occurred and can trade-offs associated with this selection be demonstrated?

Table 1. Summary of life stages and associated life-history characteristics of Plantago major investigated in the present study.

Life stage	Life-history characteristic
pre-emergence	seed dormancy single seed weight
survival from germination until first flowering	germination time age at first flowering vegetative biomass at first flowering
first reproduction	number of seeds single seed weight
survival until later flowering	
reproduction at later flowering	number of seeds single seed weight

4. What environmental factors may have caused selection?
5. Can selection on specific levels of phenotypic plasticity in life-history characteristics or related traits be demonstrated?
6. At what spatial scale of environmental variability does genetic differentiation in life-history characteristics occur?

An attempt was made to answer these questions by studying the life-history characteristics, and related traits, such as plant morphology and biomass allocation, of plants from five contrasting populations of *P. major* in the Netherlands. The plants were raised either from bulk samples of seeds from the separate populations or seed collections (lines) derived from individual plants after one or more generations of inbreeding. They were studied in reciprocal sowing or transplant experiments and in experiments in a greenhouse. When phenotypic plasticity is quantified for sets of plants that are genetically approximately identical (e.g. inbred lines), genetic variation in phenotypic plasticity is manifested as "genotype" X environment interaction (Falconer 1981). When these sets of plants are not genetically identical (e.g. population-bulk samples), then the environmental

component of the variation in a plant characteristic represents the average plasticity of the genotypes present (Rice and Bazzaz 1989).

The subsequent chapters of this thesis deal with the following case studies. Chapter 2 explores whether variation in life-history characteristics and related traits, between three populations of *P. major*, is based on genetic differentiation or rather induced by the environment. Chapter 3 presents a study on the effect of seed size on germination pattern and competitive ability within two of the aforementioned populations. This study concentrates on the question whether there are costs, in terms of lower competitive ability or a lower number of seeds produced, associated with a specific seed size. In chapter 4 the relationship between age and size at first flowering and relative fitness is investigated in five different populations. In this chapter attention is also paid to a trade-off relationship; in this case whether in specific environments costs in terms of fitness are associated with either relatively early or relatively late flowering.

The next four chapters deal with the variance in life-history characteristics within a single population on a former beach plain. The main objective of these studies is to indicate whether within this population differences in life-history characteristics and in plasticity in these properties could have evolved as an adaptation to small-scale patterns of abiotic environmental factors. For this purpose, Troelstra et al. (chapter 5) studied in detail the seasonal and spatial variability in a number of mainly soil chemical factors at a site of approximately 2,000 m^2. Lines of *P. major* from three subsites were studied in chapters 6, 7 and 8. Differences in selection regimes are related to genetic variation in morphology and life-history characteristics between lines from different origins (chapter 6). Chapter 7 presents results from an experiment to analyse the influence of nutrient supply and waterlogging on growth, morphology and flowering of the lines and to correlate plant responses to these experimental factors, to their performances at the three subsites. Chapter 8 explores the possibilities that within the beach-plain population genetic differentiation for specific levels of phenotypic plasticity in seed-yield components has occurred.

Finally, in chapter 9 some general aspects of variation in life-history characteristics of *P. major* are discussed in relation to environmental variability.

The Plantago-project: a multidiscipinary research approach

The study presented in this thesis was initiated as part of a

multidisciplinary research project on the relationship between demographic, physiological and genetic properties of *Plantago* species and the characteristics of their environment (Van der Aart 1985). The primary aim of the research project, in which five species of the genus *Plantago* were studied, was to determine which characteristics of a plant are important for its adaptation to its environment. Over the years the basic interest has shifted from a comparison between the species to a comparison between populations of one species or even to the significance of variation within populations. Some results with respect to the breeding system, potentials of gene flow and to variation in plant characteristics between and within populations of *P. major* will be reviewed shortly.

P. major is a self-compatible wind-pollinator with a high self-fertilization rate (Van Dijk 1985). This author analysed the variation in allozymes in relation to variation in other plant characteristics within this species. Two subspecies were distinguished: subspecies *major* and subspecies *pleiosperma* Pilger. Compared to the former subspecies, winter mortality in subspecies *pleiopsperma* is generally very high. It was suggested that differences in allozymes between these subspecies are caused by chromosomally linked loci determining morphology and some life-history characteristics. Both the high rate of self-fertilization and the relative low levels of gene flow (Van Dijk estimated the mean gene transport per generation within a population of *P. major* ssp. *major* to be only 0.11-0.35 m) were suggested to be the cause of a relatively high degree of genetic differentiation between populations. Within subspecies *major* two ecotypes were described: the road-side type, which is trampling resistant (Blom 1979), and the lawn type, adapted to mowing and grazing. Wolff (1988) compared morphological and electrophoretic variability within and between populations of *P. major* that were also investigated in the study presented in this thesis. She found no agreement between both kinds of variabilities over these populations. She concluded that other forces than historical events, random drift or founder effect, had at least partly influenced morphological characteristics in a different way than allozymes. Natural selection on morphological traits may be the explanation for this lack of agreement.

Kuiper (1984) demonstrated differences in phenotypic plasticity in physiological characteristics between inbred lines of *P. major*. Ion-stimulated ATP-ase activity and root respiration of plants of subspecies *pleiosperma* could be adjusted better to a change in mineral nutrition than of plants from subspecies *major*.

Lambers and Dijkstra (1987) did a physiological analysis of genetic variation in relative growth rate between inbred lines of subspecies *major* and *pleiosperma*. They concluded that higher relative growth rates of plants of subspecies *pleiosperma* are associated with a lower allocation of

dry weight per leaf area, whereas the net assimilation rate and the photosynthesis did not decrease proportionally.

Any study designed to relate variation in life-history characteristics to environmental variability ideally requires to intersect the fields of the plant population ecology, genetics and ecophysiology (cf. Dirzo and Sarukhan 1984). Ecological phenomena may determine genetic changes but may also themselves be modified by the changing composition of populations. In addition, the underlying mechanism of plant responses to the environment will be based on ecophysiolocal processes, e.g. allocation patterns. Participation within the *Plantago*-project clearly provided a stimulating possibility to work at the interface of these three disciplines.

CHAPTER 2

PLASTICITY IN LIFE-HISTORY TRAITS OF PLANTAGO MAJOR L. SSP. PLEIOSPERMA PILGER

L.A.P. Lotz and C.W.P.M. Blom

Summary. Plasticity in life-history characteristics was investigated in three populations of *Plantago major* L. ssp. *pleiosperma* Pilger, a self-compatible, wind pollinated species with a high self-fertilization rate. The populations studied were selected for their marked differences in biomass accumulation and habitat characteristics such as nutrient availability and interspecfic interaction. Plants, raised from seeds collected at three sites, were grown in a greenhouse at three nutrient levels. In addition, a reciprocal transplant experiment was carried out. In both experiments variances in variables of growth and reproduction were largely due to environmental factors. Besides this overall result, population and population x environment interaction effects existed for most of the variables. Differences in plasticity between populations were further analysed. In the greenhouse experiment plants from a river-side population showed a high degree of plasticity in reproductive effort, whereas plants from two other populations, one series from a beach plain and the other from a salt meadow, showed a high degree of plasticity in shoot-root ratio. Plasticity in biomass allocation to either vegetative or generative parts is suggested to be an important response to selective forces related to either interspecific competition or temporal variability.

INTRODUCTION

Life-history traits such as growth rate, precocity, reproductive effort as well as the phenotypic plasticity of these traits are subject to natural selection (Bradshaw 1965, Stearns 1977, Lacey et al. 1983). Therefore, life-history traits of an organism may be related to characteristics of the

habitat. Knowledge of this relationship is fundamental for understanding the adaption of organisms to their environment. In this respect it is important to question whether variation in life-history traits between populations is based on genetic differentiation or induced by the environment.

Phenotypic plasticity (Bradshaw 1965) can be studied in experiments on plants of a single genotype, e.g. inbred lines, or can otherwise be approximated in experiments on plants grown from seeds sampled in the population (e.g. Antonovics and Primack 1982, Blom 1983). This last method was also used in the experiments described in this paper. *Plantago major* L. is a self-compatible wind pollinator with a high self-fertilization rate (Van Dijk 1984). Inbred lines of four populations of *P. major* were studied by Kuiper (1982, 1983), who in water-culture experiments demonstrated that physiological characteristics, such as ion-stimulated ATP-ase activity and root respiration of plants of *P. major* ssp. *pleiosperma* Pilger could be adjusted better than of plants of *P. major* ssp. *pleiosperma* to a change in mineral nutrition. Poorter and Lambers (1986) suggest that this high phenotypic plasticity of plants of *P. major* ssp. *pleiosperma* is related to a greater competitive ability for nutrients in an artificially fluctuating environment.

In the present study both growth and reproduction were examined in three populations of *P. major* ssp. *pleiosperma*, a subspecies of ruderal habitats (Mølgaard 1976). Given the evidence of phenotypic plasticity of *P. major* ssp. *pleiosperma* in water culture (Kuiper 1982, 1983) it was hypothesized that differences in growth rate and generative development between the populations of *P. major* ssp. *pleiosperma* are determined by environmental factors, rather than by genetic differentiation. This hypothesis was tested both in a greenhouse experiment, with different levels of mineral nutrition as well as in a reciprocal transplant experiment of plants in natural habitats. Three populations which differed markedly in biomass accumulation and habitat characteristics, were selected.

MATERIALS AND METHODS

Study sites

The investigated populations are located in the Netherlands: population Angeren, situated in the centre of the Netherlands on the banks of the river Rhine, and two populations 12 km apart on the south-west coast, one

at Oostvoornse Meer and the other at Kwade Hoek. A survey of allozyme variation in these three populations was made by K. Wolff (unpublished results). Frequencies of alleles specific to the subspecies correspond with frequencies described by Van Dijk and Van Delden (1981) for subspecies *pleiosperma*. Nutrient levels of the soil and measures of some life-history characteristics in the field are summarized in Table 1.

The study site Angeren is a frequently inundated sandy river bank, moderately grazed by cattle. Compared to the other sites, the soil is quite rich in total phosphate. Higher plant cover is low (± 30%) and the individual plants, having an annual life form, are characterized by rapid growth and reproduction.

The Oostvoornse Meer site is a former beach plain, embanked in 1965. In winter it is frequently inundated by rain water. The soil is low in total nitrogen and total phosphate and there is virtually no grazing. The

Table 1. Estimates of nutrient levels (soil 0-15 cm) and measurements (means ± S.D., n=9-23) of growth and reproduction of plants of *P. major* ssp. pleiosperma at three study sites. Soil samples were taken in July 1983 and analysed after Troelstra et al. (1981). Relative growth rates of the shoots were calculated from estimates of the biomass at monthly intervals between 31 June and 28 August (Angeren) and 20 April and 26 August (Oostvoornse Meer and Kwade Hoek) in 1983

	Angeren	Oostvoornse Meer	Kwade Hoek
Soil pH - H_2O	8.0	8.3	7.2
Soil org.matter (%)	2.4	1.3	26.4
Soil tot.N ($g.m^{-2}$)	200	115	710
Soil tot.P ($g.m^{-2}$)	190	42	73
Soil Cl ($Eq.m^{-2}$)	6.4	1.1	21.0
RGR shoot (g/g dry)	0.069	0.021	0.023
	± 0.021	± 0.006	± 0.008
Dry weight fructiferous plants			
shoot (g)	0.81	0.07	0.56
	± 0.68	± 0.09	± 0.40
spike + scape (g)	0.76	0.02	0.29
	± 1.49	± 0.01	± 0.18

vegetation is species-rich. Higher plant cover is low (+ 30%). Plants, mostly short-lived perennials, are characterized by a low growth rate and a low biomass.

The Kwade Hoek site is a salt meadow, extensively grazed by cattle and occasionally flooded by sea water in winter. The soil is rich in organic matter. Level of total phosphate is intermediate and levels of total nitrogen and chloride are high, compared with the other sites. The vegetation is dense and highly productive. At this site the plants of *P. major* ssp. *pleiosperma* have perennial life forms.

In 1981 and 1982 seeds were collected from several plants at the study sites.

Greenhouse experiment

On 29 April 1983 ten seeds were sown per pot (0.75 l) in silver-sand. For each population 72 pots were used. At the start of the experiment three different nutrient levels were created with a 1/2 Hoagland solution (Smakman and Hofstra 1982). The amount of minerals is indicated by the amount of Hoagland solution supplied per pot: treatment L(ow), no supply; I(ntermediate), 0.24 l; treatment H(igh), 0.48 l. Soil moisture was kept at 20 per cent by weight. Day temperature was about 25 °C and night temperature about 20 °C.

After seedling emergence the number of plants was ramdomly reduced to one per pot. On days 20, 34 and 48 after sowing, six plant of each treatment were harvested. Fresh weights and dry weights (24 h at 70 °C) of shoot, root, and, if present, spike + scape were determined. The numbers of plants flowering on day 48 after sowing, were counted. After each harvest the remaining plants received an amount of Hoagland solution, which compensated for the uptake of minerals by the plants. Uptake of minerals was estimated by multiplying the dry weight of the harvested shoot and root by their N content, (estimated to be 3500- and 3000 mmol N/kg dry weight, respectively, on the basis of separate experiments). The remaining plants were harvested when they were reproductively mature, at 91 days (treatment I and H) and 119 day (treatment L) after sowing. The following variables were measured: fresh and dry weights of the leaves, of spike + scapes, and of roots, leaf area, weight and number of mature seeds produced by each individual plant.

Leaf material was analysed for concentrations of K, H_2PO_4 and N_{org} as described by Troelstra (1983). Shoot-root ratio was computed as fresh weight of leaves per fresh weight of roots. Reproductive effort was computed as dry weight of seeds per dry weight of leaves and roots.

On each harvest data numbers were assigned randomly to all individual

plants of each population and treatment. Relative growth rates were computed by linear regression on time series of log-transformed biomasses of the shoot of harvested plants of corresponding numbers.

Reciprocal transplant experiments

Seeds of the populations investigated were sown in moist dune sand in the greenhouse; temperatures were as mentioned above. When about 15 days old, the seedlings were exposed to outdoors conditions for several days before being planted in the field. The plants were randomly selected and transplanted into the three study sites; 48 seedlings of each population were planted alternately in a grid at Angeren and, 72 both at Oostvoornse meer and Kwade Hoek. The shortest distance between the plants was 0.10 m. The average shoot dry weight of the seedlings (n=10) at the time of transplanting was for the population Angeren 0.8 mg (S.D. 0.5), Oostvoornse Meer 1.5 mg (S.D. 0.3) and Kwade Hoek 2.0 mg (S.D. 1.0). The plants were transplanted on 31 May 1983 at Oostvoornse Meer and on 1 June at Kwade Hoek. Because of inundation of the banks of the river Rhine the afore-mentioned seedlings could not be used at the Angeren transplant site. Seedlings of a new, identical preculture were planted at Angeren on 5 July.

Every fortnight biomass of shoots of all individual plants was estimated by multiplying the number of leaves (N) by the length (L) and the width (W) of the largest leaf. The correlation between the dry weight of the shoot and the product N*L*W was determined in a separate experiment. The regression coefficient and the correlation coefficient were for population Angeren 0.15 and 0.951 (n=21, $P<0.0001$), for population Oostvoornse Meer 0.15 and 0.879 (n=14, $P<0.0001$) and for population Kwade Hoek 0.12 and 0.932 (n=31, $P<0.0001$). The plants with spikes were counted between 20 and 22 September 1983. The spikes were all harvested and the dry weights of both spikes and seeds were determined. Data of plants which had died during the experiment were excluded from determining variables of growth and reproduction. The number of plants of each population that survived at a site was at least 14.

Statistical analysis

Data were processed on the CYBER 170/76 of the State University of Groningen using S.P.S.S. subprogram MANOVA (Nie and Hull 1981). Tests for polynomial trends were based on the error mean squares of the whole analysis of variance (d.f.=45). Differences in polynomial coefficients

between populations were examined by means of t-tests. Because of simultaneous comparisons over three populations, in these t-tests 99 per cent confidence limits were considered, employing the Bonferroni inequality (Miller 1981).

RESULTS

Greenhouse experiment

Figure 1 shows the results obtained in the greenhouse experiment; a statistical analysis of the effects of mineral nutrient supply and population is summarized in Table 2. At the end of the experiment variances of the dry weight of the shoot, R.G.R. of the shoots, dry weight of seeds, reproductive effort (dry weight of seeds per dry weight of plant), and the number of seeds, were mainly due to the nutrient supply. Besides this, variance components due to population and interaction of nutrient supply x population were also present for most of the variables denoting vegetative and generative development. Variance of the R.G.R. of the shoots was explained by the supply of the nutrients only. With respect to dry weight and number of seeds the variance components due to population and the two-way interaction were very small. Contrary to this, the importance of population in explaining variance was relatively large for shoot-root ratio, leaf-area ratio and specific seed weight.

In order to investigate the population specificity of variation induced by mineral nutrient supply, polynomial trends in nutrition were studied within each population separately. The linear coefficients are presented in Table 2. Variables having a significant component of variance for nutrient supply x population interaction were selected for these trend analyses. The dry weight of the shoot of plants of the salt meadow population Kwade Hoek was most strongly influenced by nutrient supply (Fig. 1). The linear coefficient of this variable differed significantly between population Kwade Hoek and the other populations. Shoot-root ratios were very low for plants from the Angeren population (Fig. 1) and did not differ significantly between treatments, whereas shoot-root ratios of both Oostvoornse Meer and Kwade Hoek plants were quite strongly influenced by the nutrient supply (Table 2). In contrast to plants of other populations leaf-area ratios of Kwade Hoek plants differed significantly between treatments. The plants of this salt meadow population had lower leaf-area ratios when the nutrient supply was high than when it was low (Fig. 1).

Fig.1. The effect of the mineral nutrient level in silver-sand on vegetative and generative development of plants from three populations (ANG Angeren, OM Oostvoornse Meer and KH Kwade Hoek) of Plantago major ssp. pleiosperma. Means (±SD, n=6 represent fully reproductive plants at 91 (treatment I and H) or 119 days (treatment L) after sowing. L Low, no Hoagland solution supplied; I Intermediate, 0.24 ml and H High, 0.48 ml Hoagland solution added

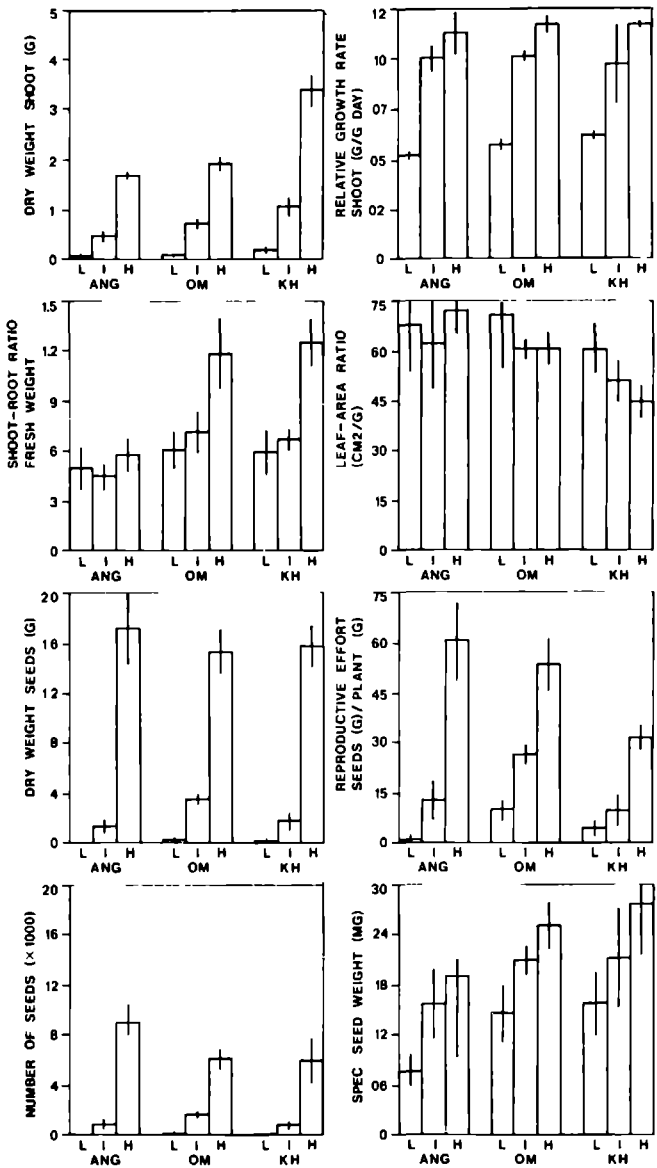


Table 2. Effect of mineral nutrient supply on vegetative and generative development of plants of three populations of Plantago major ssp. pleiosperma grown in the greenhouse. Analysis of variance. Sum of squares are given as proportion of total variance (100%). Interaction of nutrition x population was partitioned in a trend analysis. Tests for trends were based on the error mean squares (d.f.=45) of the whole experiment. Linear coefficients followed by the same letter are not significantly different (t-test).

Dependent variable	Components of variance Independent variables			Trend analyses Linear coefficients		
	Nutrition	Population	Two-way interactions	Angeren	Oostvoornse Meer	Kwade Hoek
Vegetative development						
dry weight of shoots[1]	88.9 ***	7.8 ***	2.3 ***	0.29[a] ***	0.30[a] ***	0.41 [b] ***
RGR (shoots)	90.9 ***	1.5 ns	1.5 ns	-	-	-
Shoot-root ratio (fresh weight)	44.3 ***	27.3 ***	13.8 ***	0.06[a] ns	0.42[b] ***	0.47 [b] ***
Leaf-area ratio[1]	8.5 *	30.3 ***	11.5 *	0.03[a] ns	-0.04[ab] ns	-0.09 [b] ***
Generative development						
dry weight of seeds[1]	97.1 ***	0.2 *	1.1 ***	0.31[a] ***	0.28[a] ***	0.29[a] ***
reproductive effort	77.0 ***	8.6 ***	8.3 ***	0.42[a] ***	0.31[b] ***	0.19[c] ***
number of seeds[1]	92.0 ***	0.7 **	2.7 ***	0.70[a] ***	0.56[b] ***	0.56[b] ***
specific seed weight	49.1 ***	25.6 ***	1.0 ns	-	-	-

[1] Analysis was performed after log transformation. ns not significant; * P<0.05; ** P<0.01; *** P<0.001

Nutrient supply effects on the reproductive effort differed significantly between the three populations. The linear coefficient of trend in nutrient supply was highest in the case of Angeren plants (Table 2). When the nutrient supply was low these riverside plants invested a very low poportion of biomass in seed production, whereas the reproductive effort was more than 0.6 at the high nutrient supply (Fig. 1). Reproductive effort of plants from population Kwade Hoek was influenced less by nutrient supply than that of the other populations. For these salt-meadow plants this ratio was lower for all treatments than that of Oostvoornse Meer plants. With regard to the number of seeds, the plants from population Angeren were also most strongly influenced by nutrient supply, the number of seeds ranging from almost zero to 16,000 per plant. Specific seed weight of plants from population Angeren was lowest for all treatments (Fig. 1, Table 2).

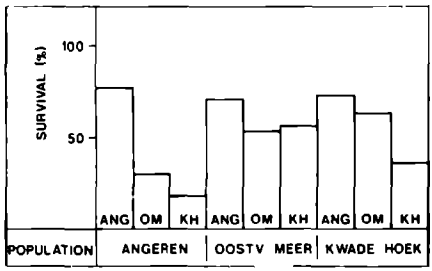
Seed production of all plants was determined at full maturity, but during the experiment there were differences in precocity, between treatments as well as populations. On day 48 after sowing all plants of the three populations with a high nutrient supply flowered. At the intermediate nutrition level 5 plants (42%) from population Angeren, 12 plants (100%) from population Oostvoornse Meer and no plants from population Kwade Hoek flowered on day 48. At this intermediate treatment the frequencies of flowering differed between populations (Chi^2=24.3; d.f.=2, P<0.001). At the lowest nutrient level no plants flowered on day 48.

Concentrations of K, H_2PO_4 and N_{org} in leaf material were determined to investigate which nutrient was the limiting factor. In all leaves concentrations of H_2PO_4 were less than 30 meq.kg^{-1} d.m., which may be considered extremely low compared to results of analyses of collected field material, ranging from 60 to 168 meq.kg^{-1} d.m. (Troelstra et al. 1983). Contents of K, ranging from 561 to 1062 meq.kg^{-1} d.m., and N_{org}, ranging from 822 to 1263 mmol.kg^{-1} d.m., did not differ from plants sampled in the field (Troelstra et al. 1983, Troelstra pers. comm.).

Reciprocal transplant experiment

The proportion of transplanted plants which survived during the experiment, depended on the study site (Fig. 2, Chi^2=46.5, d.f.=2, P<0.001). Survival was highest at Angeren and lowest at Kwade Hoek. Angeren plants showed a higher survival rate in their own habitat than at the coastal sites (Chi^2=39.26, d.f.=1, P<0.001). At Kwade Hoek only 19% (14 plants) of this population was still alive after 113 days. Differences in suvival rate of plants of populations Oostvoornse Meer and Kwade Hoek at the sites Oostvoornse Meer and Kwade Hoek were not significant.

Fig 2. Survival of plants from three populations of Plantago major ssp. pleiosperma in a reciprocal transplant experiment. Study sites are indicated as ANG Angeren, OM Oostvoornse Meer, KH Kwade Hoek. Survival was determined after 79 (site Angeren, n=48 plants per population) or 112 days (sites Oostvoornse Meer and Kwade Hoek, n=72 plants per population)



Data of vegetative and generative development of transplanted plants are presented in Fig. 3 and statistics are summarized in Table 3. Variances in the dry weight and in the relative growth rate of the shoot were mainly explained by the study site. On the sandy river bank of site Angeren the biomass of plants of all populations was highest, in spite of being transplanted late in the season. Components of variance due to the population and the two-way interaction were small or absent.

At Angeren plants of all populations had flowered and set seed at the end of the experiment. At this river-side flowering frequencies differed between populations (Chi^2=8.5, d.f.=3, $P<0.05$). Population Oostvoornse Meer had the highest proportion of plants that flowered (61%), while population Kwade Hoek had the lowest (19%). At the Oostvoornse Meer and Kwade Hoek sites only plants of population Oostvoornse Meer flowered (38 and 13%, respectively). About 7% of the total variance in production of spikes and seeds at site Angeren was due to the population (Table 3).

DISCUSSION

Selective forces which lead to the evolution of life-history traits may be investigated in studies on variation between (Stearns 1977) or within (e.g. Schmitt 1983) populations. Following the first approach, in a greenhouse

Fig. 3. Vegetative and generative development in a reciprocal transplant experiment with plants from three populations into three sites (ANG Angeren, OM Oostvoornse Meer and KH Kwade Hoek) of Plantago major ssp. pleiosperma. Means (±SD, 14≤n≤46) are represented

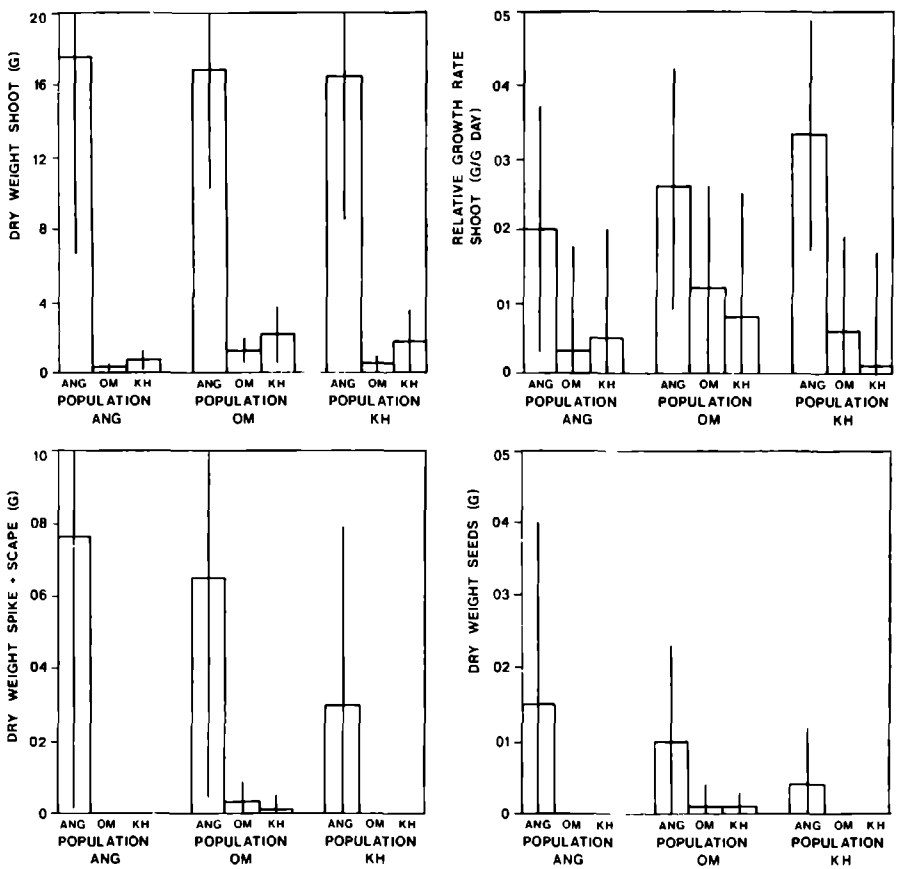


Table 3 Vegetative and generative development in a reciprocal transplant experiment with plants of three populations of Plantago major ssp pleiosperma Results of a two-way (dry weight of shoots and RGR of shoots) and a one-way analysis of variance (dry weight of spikes and seeds) Sum of squares are given as proportion of total variance (100%) Data of plants which died during the experiment were excluded

Dependent variable	Independent variables		Two-way interaction
	Site	Population	
Dry weight of shoots[a]	84 6 ***	1 3 ***	0 8 ***
RGR (shoots)	27 9 ***	2 3 **	2 1 ns
Dry weight of spikes[a]	-	7 0 *	-
Dry weight of seeds[a]	-	7 7 *	-

ns not significant
* P<0 05, ** P<0 01, *** P<0 001
[a] Analysis was performed after log transformation

experiment with three different populations of *P. major* ssp. *pleiosperma* vegetative and generative development were strongly affected by the supply of nutrients. Besides this overall result, most of the variables showed population and nutrient supply x population effects. These interactions may reflect differences in plasticity between populations. For example, shoot-root ratios of plants from both the nutrient-poor beach plain Oostvoornse Meer and the salt meadow Kwade Hoek depended strongly on nutrient supply, whereas the treatments made no difference to the shoot-root ratios of plants from the river bank at Angeren. The high amount of variation in shoot-root ratios of plants from Oostvoornse meer and the constancy in this ratio for Angeren plants are remarkable. According to Chapin (1980), species from nutrient-poor habitats normally demonstrate less phenotypic plasticity in shoot-root ratios than species from nutrient rich habitats. Obviously, this generalization does not fit between-population variation in plasticity for shoot-root ratios in *Plantago major* ssp. *pleiosperma*. Contrary to plants from the other populations nutrient supply had the greatest effect on reproductive effort of plants

from the river-side. At the low nutrient level plants from population Angeren produced almost no seeds, whereas at the high level about 60% of plant biomass was invested in seed production. This high percentage corresponds to maximum values mentioned by Soule and Werner (1981) in a review of reproductive efforts of 13 species. Even under nutrient rich conditions the reproductive effort of plants from the salt-meadow Kwade Hoek were low.

In the reciprocal transplant experiment variances in vegetative and generative development also depended mainly on the environment, i.e. the study site. Nevertheless, in this field experiment, too, there are indications that plants from the populations under study differ in plasticity, e.g. in the survival of transplants and in precocity. Only plants from population Oostvoornse Meer produced seeds at all study sites.

The overall pattern of relative importance of the environment in determining differences in life-history characteristics, which was found in the present experiments as has been hypothesized, is in accordance with the high amount of phenotypic plasiticity in a whole set of physiological characteristics reported by Kuiper (1982, 1983) for *P. major* ssp. *pleiosperma* grown in culture solution.

Besides, the present results demonstrated a variety in degree of phenotypic plasticity in some life-history characteristics between *P. major* ssp. *pleiosperma* populations which may be related to differences in natural habitats. Plants from the nutrient rich, frequently inundated river bank at Angeren showed a higher plasticity in reproductive effort. Hickman (1975) and Primack and Antonovics (1981) also reported high plasticity in reproductive allocation in plants from harsh, open environments. On the contrary, plants from populations Oostvoornse Meer and Kwade Hoek, both with (short-lived) perennial life histories, exhibit a higher degree of plasticity in shoot-root ratio. In these environments in which competition from neighbouring plants is an important aspect, plasticity in patterns of biomass distribution to shoot and roots may be of adaptive value. By changing shoot-root ratios a plant may adjust its ability to compete either for light or for nutrients in the process of vegetation development and thus consolidate its pre-reproductive development.

The plasticity in reproductive effort of plants from the nutrient-poor beach plain population Oostvoornse Meer is intermediate between that of plants from populations Angeren and Kwade Hoek. In this population selection for early flowering and high reproductive efforts, may have been possible even when the nutrient supply was limited.

The results of this study demonstrate a variety in degree of phenotypic plasticity in life-history characteristics in *P. major* ssp. *pleiosperma* populations from different sites. It is suggested that phenotypic plasticity expressed in either vegetative or generative development is an important

response to selective forces as interspecific competition or temporal variability.

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CHAPTER 3

THE EFFECT OF SEED SIZE ON GERMINATION PATTERN AND COMPETITIVE ABILITY OF PLANTAGO MAJOR L. SSP. PLEIOSPERMA PILGER

L.A.P. Lotz and H. Coops

Summary. Variability in seed size was studied in two contrasting populations of *Plantago major* L. ssp. *pleiosperma*. Seeds from a population of a river bank, a highly unpredictably variable environment, were on average smaller than those from a population of a shore meadow, an environment with a high level of competition. The effect of seed size on germination, establishment and reproduction was studied, using seeds of different size classes from both populations. In a climate cabinet relatively small seeds showed a higher level of dormancy than large seeds. Moreover, irrespective of the size, seeds from the river-bank population had a considerably higher degree of dormancy than those from the shore meadow.

Germination, growth and reproduction were studied in a greenhouse experiment in which seedlings were grown in different densities of *Festuca rubra* L. and in a reciprocal sowing experiment. Seedlings from the shore-meadow population had, on average, a higher final plant weight and reproductive output. No effects of seed size on these traits could be demonstrated. The higher competitive ability of the shore-meadow plants was probably caused by a higher biomass investment in photosynthetic tissue.

The native plants proved to be better adapted to their own environmental conditions than alien plants for several life-history characteristics (e.g. dormancy, seedling growth, age at first flowering). Inspite of the observed differences in seed size between both populations, adaptive value of relatively large seeds with respect to survival of the seedling until reproduction was not found. It is hypothesized that a cost- in terms of fitness - of producing small seeds in the shore-meadow population is more related to the germination pattern of the seeds, i.e. a too long period of dormancy in this environment with a high level of plant-plant interference, than to the competitive ability of the seedling.

For establishment of seedlings in specific environments two characteristics of seeds are generally thought to be important. The first characteristic is seed size and the second seed dormancy. Comparing the seed size and establishment of different species, it has been hypothesized that in a closed vegetation only relatively large seeds contain sufficient stored energy to let the seedling survive until it reaches high enough into the canopy to support itself photosynthetically (e.g. Salisbury 1942, Harper 1977, Gross 1984, Thompson 1987). However, Thompson (1987), in a review of many experimental results, showed that the relationship between seed size and competitive ability is not unequivocal and at least partly depends on the availability of resources and the species studied.

It is generally believed that there is a trade-off between the number and the size of offspring an individual plant is able to produce (Stebbins 1971, Smith and Fretwell 1974, Wilbur 1977, Primack 1978, Lloyd 1987). In fluctuating environments a plant's fitness may depend more on the number of offspring that escape unfavourable growth conditions than upon seed size and thus, the growth potential of individual progeny.

The second important characteristic of seeds is their capacity to remain dormant in the soil. It has been suggested (e.g. Cohen 1966, Jain 1979, Venable and Lawlor 1980) that variation in seed dormancy, that in general helps to maintain a reserve of viable seeds in the event of catastrophic environmental conditions, may be of adaptive value. There is experimental evidence that the degree of dormancy may depend on seed size (Cook 1980, Weis 1982, Zimmerman and Weis 1983). In his review Cook (1980) concluded that selection for persistence in the soil may favour a decrease in seed size, whereas the seed-coat thickness does not change. The relative increase in the thickness of the seed coat will probably enhance the structural strength the endosperm needs to develop to break it, in order to emerge. In addition, generally small seeds have a greather chance than larger seeds of getting buried in the soil and of becoming part of a persistent seedbank (Thompson 1987).

Various papers have been published on effects of within-species differences in seed-size on variability in germination and seedling growth (e.g. Weis 1982, Zimmerman and Weis 1983, Stanton 1984, Stanton 1985, Van Groenendael 1985, Van Groenendael 1986, Wulff 1986 a and b). Whether seed size is subject to stabilizing selection in natural populations is questioned, because wild plants in general show marked phenotypic

plasticity and low heritability of seed size (Winn 1988, Silvertown 1989). In the present paper the effect of seed size on germination pattern and competitive ability is studied in two contrasting populations of *Plantago major* L. ssp. *pleiosperma* Pilger, located at a river bank and at a shore meadow, respectively. The vegetation of the river bank is very open. At this site plants produce many small seeds. The vegetation of the shore meadow is dense. At the latter site single seed weight of *P. major* ssp. *pleiosperma* is higher than at the river bank. The differences in single seed weight between both populations are, partially, genetically determined (Lotz and Blom 1986).

Plantago major is generally not able to establish itself from seeds in a closed vegetation (Pons and Van der Toorn 1988, Van der Toorn and Pons 1988). Moreover, Blom (1987) suggested from results of a long-term demograhic study that fluctuations in numbers of *P. major* ssp. *pleiosperma* plants at the aforementioned shore meadow are due to poor establisment of seedlings when there are no open sites in the vegetation. At this study site, seeds of *P. major* ssp. *pleiosperma* germinate primarily in gaps, caused by trampling cattle (personal observation L.A.P. Lotz). After germination these seedlings will experience high interference of grasses that also colonize these gaps.

The purpose of this study is to test whether seed size positively affects the competitive ability of *P. major* ssp. *pleiosperma* and, therefore, to demonstrate a potential cost - in terms of fitness - of relatively small seeds for a mother plant. The following questions will be addressed:

1. Do seeds of different size classes vary in patterns (in time) of germination?
2. Do seedlings from relatively large seeds have a higher growth rate and reproduction when grown between grasses than seedlings from relatively small seeds?
3. Are seedlings better adapted to their own environment (in this study characterized by high unpredictability or a high degree of competition, respectively) and are these differences in adaptation related to different seed sizes?

We tried to answer these questions by comparing effects of seed size in both populations and in both habitats. We studied germination in a climate cabinet and seedling establishment and growth until reproduction, in a reciprocal sowing experiment and in a greenhouse in which seedlings were grown at different densities of *Festuca rubra* L. To indicate the effect of seed size on germination pattern and competitive ability of genotypes within both populations we used seeds from various lines in the greenhouse experiment.

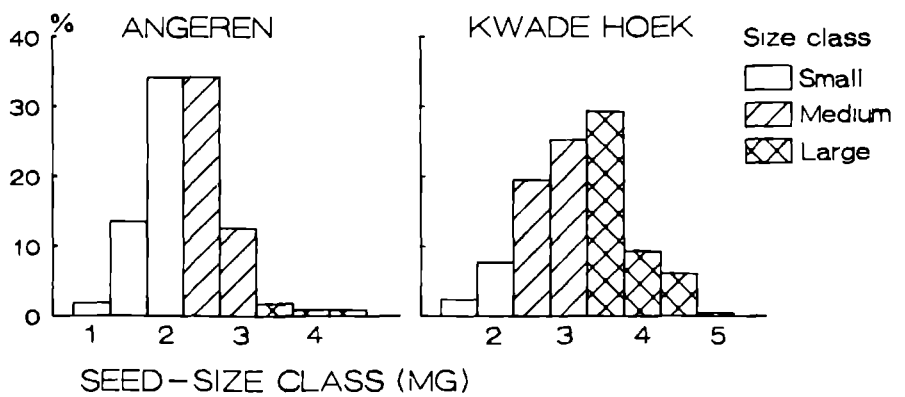
MATERIALS AND METHODS

Populations, study sites and seed colections

The investigated populations of *P. major* ssp. *pleiosperma* are located in the Netherlands: population Angeren is situated in the centre of the Netherlands on the sandy banks of the river Rhine, and population Kwade Hoek on a shore meadow on the south-west coast. Differences in habitat and life-history characteristics between both populations were studied by Lotz and Blom (1986). The habitat of the river bank population is characterized by a high seasonal variability, due to flooding in winter, spring and autumn. In summer the site is moderately grazed by cattle. Higher plant cover is low (c. 30%). Plants of this population have an annual or a short-lived perennial life cycle, a short vegetative period and produce a high number of seeds. The shore meadow is extensively grazed by cattle. At this site the vegetation, with e.g. *Festuca rubra*, is dense and highly productive. In contrast to the plants of the river bank, the coastal plants have a perennial life cycle and a long vegetative period.

Bulk samples of mature seeds from each population were collected in October 1984. In addition, seeds from ten individual plants were collected in October 1985 and 1986. Seeds from the river population were on average smaller than seeds from the shore meadow (Fig. 1; $F_{1,356}$=15.6,

Fig 1 Size distribution of seeds in bulk samples from two populations of P major ssp pleiosperma Angeren (river bank, n=111) and Kwade Hoek (shore meadow, n=246). The size classes used in the experiments are indicated



P<0.001). Three classes of seed size were defined: small (<0.2 mg), medium (0.2-0.3 mg), and large (>0.3 mg). The seeds from the river population were mainly in the small and medium class, seeds from the shore-meadow population in the medium and large class (Fig. 1).

In autumn 1985 six plants in the reproductive phase, were randomly sampled from each site, defoliated and transplanted into pots with potting compost. The plants were grown until reproduction in a greenhouse (20 °C, artificial lighting: light-dark period 16 h - 8 h). Cross-pollination was prevented with paper bags when the plants were flowering. Lines were obtained by collecting mature seeds from each plant separately.

Germination in a climate cabinet

Seeds from the "1985" samples were stored at 4 °C in the dark for one year. In October 1986, these "1985" seeds and the "1986" seeds, harvested one day before the start of the experiment, were spread out in petri-dishes with moist filter paper and incubated for 28 days in a climate cabinet (20 °C, light regime 1 W/m^2, light-dark period 12 h - 12 h). The small and medium seeds of the river-bank population were used and for the shore-meadow population, the medium and large class seeds. Per size class per line one petri-dish was used (with each c. 40 seeds). The incubation temperature of 20 °C is the lowest temperature at which seeds of *P. major* germinate (Blom 1978, Soekarjo 1983). During the incubation period germinated seeds (defined as seeds of which the seed coat was broken and the hypocotyl visible) were counted daily and removed. The mean germination time was computed as $(N_1 + 2*N_2 + 3*N_3 + 4*N_4)/ (N_1 + N_2 + N_3 + N_4)$, where N_x is the number of seeds germinated in week x. To determine whether non-germinated seeds were dormant, they were pricked with a needle through the pericarp and the endosperm and incubated (in the dark, 30 °C) in a 0.1% solution of 2,3,5, trifenyl tetrazolium chloride for 48 hours. After the incubation, the seeds were intersected and the presence of formazan crystals in the embryo tissue was investigated with a stereomicroscope. These red coloured crystals demonstrate the presence of biological reducing systems (Mackay 1972). Percentage of dormancy was computed as the number of stained seeds divided by the sum of the numbers of germinated seeds and stained seeds.

Reciprocal sowing experiment

On 15 May 1985, 600 circular gaps (10 cm^2 upon the soil surface) were created with a corer in a regular pattern in the vegetation of the shore

meadow Kwade Hoek. The shortest distance between the centres of two gaps was 0.10 m. In each gap three seeds from a "1984" bulk sample (stored at 4 °C in the dark for seven months) were sown from either population Angeren (size class small or medium) or population Kwade Hoek (size classes medium or large). The triplets of seeds were sown in a completely randomized design within each site. In a sowing experiment seedlings that occur naturally can be mistaken for seedlings from seeds that were sown. In the present experiment this risk was minimized by carefully positioning the seeds in the centre of the gaps.

Every fortnight the number of seedlings of *P. major* ssp. *pleiosperma* per gap was determined. When there was more than one seedling per gap, the number of seedlings was reduced to one. Every month the survival, the number of leaves (N), and the length (L) and the width (W) of the largest leaf were determined. Leaf area was estimated by multiplying N by L by W. This product is highly correlated with the dry weight of the leaves of *P. major* (Lotz and Blom 1986).

On 7 July 1985, at the river-bank site Angeren 300 triplets of seeds were sown in a similar design to that at the shore meadow. Sowing at an earlier date was not possible due to flooding. Because of the lack of vegetation, seeds were sown directly in the sandy soil. At equal time intervals the same plant characteristics were determined as for the shore meadow. The final census date was at location Angeren 15 October and Kwade Hoek 16 October 1985. On these dates the flowering of the plants was also scored.

Competition experiment in the greenhouse

On 18 February 1986, 432 pots with quartz sand (height of pot 9 cm, contents 0.42 l) received 75 ml of a nutrient solution, containing macronutrients in twice the strength as described by Steiner (1986) and micronutrients in twice the strength as described by Smakman and Hofstra (1982). The day after, five seeds (stored at 4 °C in the dark for about a month before the start of the experiment) from each size class of six lines of each population were sown per pot. A factor "grass density" with three levels was created by sowing seeds of *Festuca rubra* cv. *koket*: no seeds ("no grasses"), 25 seeds= 0.5 seeds/ cm^2 ("low density"), 49 seeds= 1.0 seed/ cm^2 ("high density"). Prelimnary investigations led us to expect that the resulting grass densities would be simular in range as those of the shore meadow. There were six pots for each combination of line (within each population), size class and density. The pots were placed in a greenhouse (20 °C, artificial lighting: light-dark period 16 h - 8 h). During the experiment enough water was added to maintain soil moisture at c. 20% by weight. On day 51 and day 90 after sowing the initial supply of nutrients

to each pot was repeated.

On day 7 after sowing the number of seedlings of *P. major* ssp. *pleiosperma* per pot was determined. If there were more than one seedling per pot the excess were removed. Because of relatively low rates of germination only 71% pots prepared for population Angeren and 76% for population Kwade Hoek had *Plantago* plants. The resulting number of pots per combination of line, seed size and grass density ranged between 4-6. However, a few lines had only 2 replicates.

On day 50 after sowing one randomly selected *Plantago* plant for each combination of line, seed size and grass density was harvested and the following characteristics were measured: leaf angle (angle between second and third youngest leaves which are opposed to each other in the leaf rosette), leaf area, dry weights of leaves, spikes and roots. The leaf-weight ratio (LWR) of these plants was computed as the dry weight of leaves per total plant dry weight and the leaf-area ratio (LAR) as leaf area per total plant dry weight.

On day 120 after sowing the remaining *Plantago* plants were harvested and the above-mentioned plant characteristics were again determined. However, the dry weight of the *Plantago* roots could not be determined as they could not be separated from the roots of *Festuca*.

Data analysis

Differences in percentages of germination, survival, and flowering were tested by fitting log-linear models (Norusis 1986). Data on proportion of dormancy, mean germination time, growth, morphology and reproductive output (non-flowering plants excluded for dry weight of spikes) were analysed by analysis of variance. Data on proportion of dormancy were arcsin(square-root x)-transformed, data of leaf area and dry weights were log-transformed. In the germination test in the climate chamber, independent variables were: year of collection, population (replicated by ten different mother plants), and seed size; in the reciprocal sowing experiment: site, population, seed size, and the within-subject factor time; in the greenhouse experiment: grass density, population, seed size, and in the final harvest line nested within population. In order to avoid missing cells in these analyses, seed size was recoded within each population to only two size classes, "small" and "large". Non-significant mean squares were pooled with the within-cell mean squares when appropriate. Effects of the experimental factors were considered as being significant when $P<0.05$.

Table 1. Percentage of dormancy of seeds from population Angeren (river bank) and Kwade Hoek (shore meadow) in a climate cabinet after 28 days. Means are given over ten plants with each c. 40 seeds. Seeds from the 1985 samples were stored for seven months in the dark at 4 °C. Seeds from the 1986 samples were collected within 24 h before the start of the experiment. Size classes: small (<0.2 mg), medium (0.2–0.3 mg) and large (>0.3 mg).

Sample	Size class	Population	
		Angeren	Kwade Hoek
1985	small	54	-
	medium	43	11
	large	-	6
1986	small	79	-
	medium	42	2
	large	-	1

RESULTS

Germination in a climate cabinet

Taken over both years of collection and seed-size classes, seeds from population Angeren had a considerable higher percentage of dormancy than seeds from population Kwade Hoek (Table 1, $F=62.6$, d.f.=1,71, $P<0.001$). In addition, the percentage of dormancy decreased with increasing seed size ($F=5.3$, d.f.=1,71, $P<0.05$). No effect of year of collection on seed dormancy could be demonstrated. In all samples the proportion of nonviable (i.e. unstained in the tetrazolium test) seeds was less than 4%.

The mean germination time of seeds from Angeren was 1.7 and from Kwade Hoek 1.3 week (effect of population: $F=13.9$, d.f.=1,66, $P<0.001$). The variance in germination time did not significantly differ between populations. Neither effects of seed size nor of year of collection on mean germination time could be detected.

Reciprocal sowing experiment

A higher percentage of the total number of seeds that were sown at the river bank Angeren germinated, than at the shore meadow at Kwade Hoek (Tab. 2A). Although at Angeren the percentage of germination was not different between populations, at Kwade Hoek, however, seeds of population Angeren had a higher percentage of germination than seeds of the native population. At Angeren more than 90%, and at Kwade Hoek about 70% of the total number of seeds germinated within a month after sowing (Tab. 2B). No significant effect of seed size was found on the total germination during the period of observation, nor on the pattern of germination in time.

Survival of seedlings until the end of the growing season was considerably lower at Kwade Hoek than at Angeren (Tab. 2C). At Angeren the percentage of survival of seedlings was not significantly different between populations. However, at Kwade Hoek survival of seedlings from population Angeren was lower than from the native population (Tab. 2C). No significant effect of seed size was found on the survival rate of seedlings at any site.

In the period between the first and the second non-destructive measurments the RGR of the shoots of seedlings that had emerged within one month after sowing differed both between sites and between size classes (Tab. 2D). At Angeren the average RGR, computed on basis of the estimated leaf area, was 0.074 and at Kwade Hoek 0.061 mm^2/mm^2 day. For both populations seedlings that had emerged from smaller seeds had higher RGR than seedlings from larger seeds (the interactions between seed size, site and population were not significant). No significant effects of seed size could be detected during the other months of the observation period (data not shown).

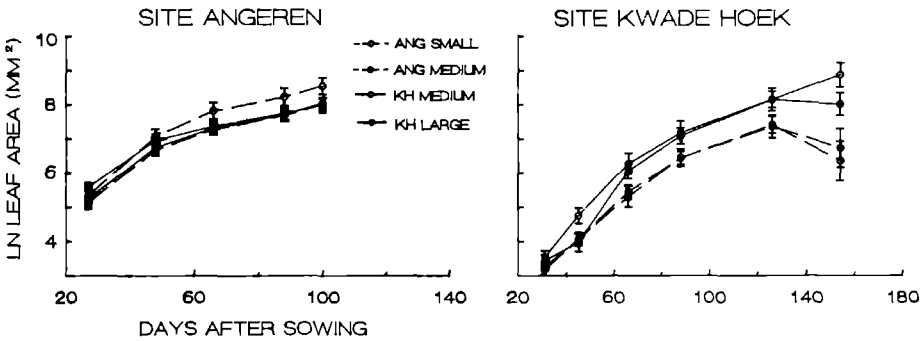
Over the whole observation period (excluding data of day 154 after sowing at Kwade Hoek) seedlings from the native population had a slightly larger leaf area at their "home" site (Fig. 2, interaction site x population: $F=4.7$, d.f.= 1,208, $P<0.05$). Only at Angeren the seedlings from Angeren seeds of the small size class had a larger leaf area (Fig.2). In contrast to this, at Kwade Hoek seedlings from the native population had a considerably larger leaf area than those from Angeren, irrespective of seed weight.

At Kwade Hoek on day 154 after sowing the difference in leaf area between both populations was even greather (Fig.2, effect of population: $F=16.3$, d.f.=1.55, $P<0.001$). At this site at the end of the growing season shoots of plants from population Angeren were more intensively grazed by slugs than plants from the native population (data not shown). Over the whole observation period effects of seed size on leaf area were not

Table 2. Germination, establishment and flowering in a reciprocal sowing experiment with seeds of different size classes from two populations of P. major ssp. pleiosperma. Observation period: at location Angeren (riverside) from 7 July until 15 October 1985, and at location Kwade Hoek (shore meadow) from 15 May until 16 October 1985. Size classes : small (<0.2 mg), medium (0.2 - 0.3 mg) and large (>0.3 mg). Effects with significant statistics are mentioned. * P<0.05, ** P<0.01, *** P<0.001.

	Size class	Site Angeren		Site Kwade Hoek		Significant effects
		population		population		
		Angeren	Kwade Hoek	Angeren	Kwade Hoek	
A. Total germination in observation period (%), n = 225	small	26.9	-	24.9	-	Site: $Chi^2_{(1)} = 19.7$ ***
	medium	29.6	23.6	21.1	16.4	
	large	-	31.1	-	18.0	Site x Population: $Chi^2_{(1)} = 4.9$ *
B. Germination within one month, expressed as % of A.	small	98.2	-	78.5	-	Site: $Chi^2_{(1)} = 55.1$ ***
	medium	96.9	93.1	69.5	70.3	
	large	-	92.7	-	73.8	
C. Survival of seedlings in observation period (%)	small	90.2	-	29.3	-	Site: $F_{(1, 128)} = 36.7$ ***
	medium	92.5	82.9	26.9	50.0	
	large	-	90.9	-	33.9	Size class: $F_{(1, 128)} = 6.6$ *
D. RGR-shoot of seedlings of B (mm^2/mm^2 day)	small	.083	-	.063	-	Site: $Chi^2_{(1)} = 14.8$ ***
	medium	.074	.072	.048	.087	
	large	-	.066	-	.039	Site x Population: $Chi^2_{(1)} = 4.3$ *
E. Flowering of plants, expressed as % of C.	small	24.3	-	11.9	-	Size class: $Chi^2_{(1)} = 4.5$ *
	medium	5.6	5.9	5.1	14.3	
	large	-	2.5	-	10.8	

Fig. 2. Leaf-area development of plants from two populations of P. major ssp. pleiosperma in a reciprocal sowing experiment: population Angeren (river bank) and population Kwade Hoek (shore meadow). Seeds were used of different size classes. Data of plants that died before the last census date were excluded.



significant.

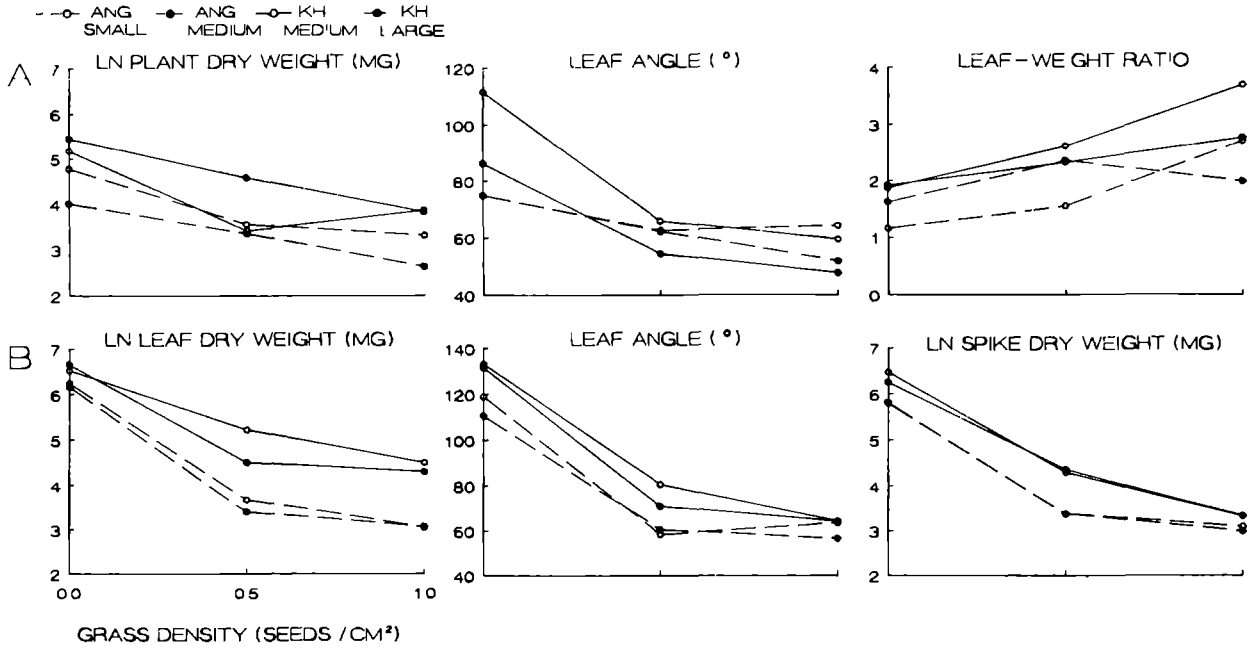
Plants that had emerged from relatively small seeds had a higher percentage of flowering than plants that emerged from relatively large seeds. (Tab. 2E). The results suggest a weak tendency that at Angeren flowering occurred less frequently than at Kwade Hoek (Chi^2=3.6, d.f.=1, P=0.06). Difference in percentage of flowering between populations was not significant.

Competition experiment in the greenhouse

On day 7 after sowing 41% of the seeds from population Angeren and 75% of the seeds from population Kwade Hoek had germinated (difference between populations: Chi^2=103.0, d.f.=1, P<0.001). Seeds of *Festuca* germinated at about the same time as the seeds of *Plantago*. No effects of seed size and grass density on percentage of germination were found.

On day 50 after sowing plant dry weight of *Plantago* was lower at higher density of *Festuca rubra* (Fig. 3A, effect of grass density: F=10.1, d.f.=2,54, P<0.001). Lines from population Kwade Hoek had, taken over all grass densities and seed-size classes, higher plant dry weights than lines from population Angeren (F=8.3, d.f.=1,54, P<0.01). On day 120 after sowing, dry weight of the *Plantago* leaves was also markedly reduced at higher grass density (Fig. 3 B, effect of grass density: F= 506.3, d.f.=2,266, P<0.001). This reduction was smaller for lines from population Kwade Hoek than for lines from population Angeren (effect of interaction grass

Fig. 3. Biomass, morphology and reproduction of plants from two populations of P. major ssp. pleiosperma: population Angeren (river bank) and population Kwade Hoek (shore meadow). Plants were derived from seeds of different size classes and grown in pots with three densities of Festuca rubra. Means were computed over six lines with one harvested plant at day 50 (A) or with 1-5 replicates harvested at day 120 after sowing (B). For dry weight of spikes only flowering plants were included.



density x population: F=15.4, d.f.=2,266, P<0.001).

Both on day 50 and 120 after sowing the angle between the opposing second and third youngest leaves of *Plantago* plants was lower in pots with *Festuca* than in pots without this grass (Fig. 3 A, B; effect of grass density: F=12.7, d.f.=2,54, P<0.01 and F=206.8, d.f.=2,248, P<0.001, respectively). The results show a non-significant tendency that this reduction of the leaf angle was stronger for lines from population Kwade Hoek than from population Angeren on day 50 after sowing (F=2.9, d.f.=2,54, P=0.06). On day 120 after sowing, taken over all grass densities and seed size classes, lines from population Kwade Hoek had a larger leaf angle than lines from population Angeren (Fig. 3B, F=6.31, d.f.=1,10, P<0.05).

The LWR increased as the grass density increased (Fig.3 A, effect of grass density: F=8,6, d.f.=2,54, P<0.001). In addition, plants from population Kwade Hoek had a higher LWR than plants from population Angeren (F=8.1, d.f.=1,54, P<0.01).

On day 50 after sowing 89% of the Angeren plants and 18% of the Kwade Hoek plants flowered in the pots without grasses (differences between populations: Chi^2=79.3, d.f.=1, P<0.001). There was hardly any flowering at this date in the treatments with grass. On day 120 after sowing the grass density treatment markedly influenced the percentage of flowering of *Plantago* plants (Chi^2=45.7, d.f.=1, P<0.001). All *Plantago* plants flowered in the absence of *Festuca*, whereas only 66% of the plants flowered in the pots with high grass density. Differences in percentage of flowering between populations on day 120 after sowing depended on the seed size (Chi^2=6.3, d.f.=1, P<0.05). These proportions were for plants derived from Angeren seed-size classes small and medium 74 and 79%, and for plants from Kwade Hoek seed-size classes medium and large 93 and 77%, respectively. Dry weight of spikes was markedly lower at higher grass density (Fig. 3 B, F=239.0, d.f.=2,206, P<0.001). In addition, lines from population Kwade Hoek had a higher dry weight of spikes than lines from population Angeren (F=6.9, d.f.=1,10, P<0.05).

Besides the aforementioned effect of seed size on percentage of flowering, no effect of seed size could be demonstrated on either the dry weight of the total plant and the leaves, the morphology or the reproductive output of *Plantago* plants.

On day 120 after sowing, significant differences between lines were found for all plant characteristics determined (data not shown). These effects that represent genetic variation within both populations, did not depend on the seed size.

Variability in patterns of germination in time

In the climate cabinet seeds from the river-bank population Angeren had a considerably higher percentage of dormancy and a longer mean germination time than seeds from the shore-meadow population Kwade Hoek. The higher percentage of innate dormancy (sensu Harper 1977) of the Angeren seeds might be of adaptive significance in the unpredictable environment of the river bank.

Within each investigated population, relatively small seeds had a higher percentage of dormancy than larger seeds. Winn (1985) demonstrated that larger seeds of *Prunella vulgaris*, a species that occurs both in woodlands and grasslands, were able to emerge in a greater variety of microsite types than small seeds. This implies that germination of small seeds of this species is more inhibited by environmental factors, i.e. induced dormancy (Harper 1977), than large seeds. Seed size did not appear to be correlated with level of dormancy in *Silene dioica* (Thompson 1981).

Pons and Van der Toorn (1988) demonstrated that germination of *P. major* seeds was inhibited by vegetation cover. This inhibition could be explained by a decrease of the red/far-red ratio of the light. The results of the germination test in the climate cabinet suggest that seeds from population Kwade Hoek, also when freshly harvested, will germinate in a recently created gap in the shore-meadow vegetation as soon as environmental conditions (e.g. red/far-red ratio, temperature and moisture) are favourable. Early germination may increase fitness of seedlings in gaps which are also colonized by grasses (Naylor 1980, Venable 1984).

The higher level of dormancy of Angeren seeds in the climate cabinet was coupled with a lower percentage of germination in the greenhouse experiment. However, similar differences between seeds from both populations in percentage of germination could not be demonstrated in the reciprocal sowing experiment. For both populations percentages of germination of seeds that were stored for one year, were considerably lower at the field sites than in the climate cabinet. Various environmental factors (e.g. predation, pathogens, low soil temperatures) may have caused these different results.

Establisment and reproduction in two contrasting habitats

The contrast between the environments of the river-bank site and the

shore meadow are clearly demonstrated in the reciprocal sowing experiment. On the river bank as soon as the water recedes, a relatively high percentage of seeds germinated in the absence of vegetation cover. Seedling survival was relatively high. However, because of the short growing season only a few plants reached the flowering stage. At the shore meadow Kwade Hoek relatively few seeds germinated in the gaps that were being colonized by grasses. Survival of seedlings was considerably lower in these gaps than on the river bank. Although at Kwade Hoek the growing season was relatively long, only few plants flowered the first season.

One might expect in both habitats a positive effect of the size of a seed on establishment and reproductive output of the seedling emerging from it. On the river bank seed size may be positively correlated with final plant biomass when seeds of different sizes produce plants with equal RGR (Choe et al 1988), and therefore might increase the probability to reproduce before the water again rises. In the gaps on the shore meadow a large seed size may result in a high initial plant weight, and therefore a high competitive ability against grasses. We tested the effect of the relative (and not the absolute) seed size in the statistics applied. Even when the effect of seed size is linear, this relative effect may depend on the factor population, because seeds from population Angeren were represented by classes "small" and "medium" and seeds from Kwade Hoek by classes "medium" and "large". Neither in the field, nor in the greenhouse experiment could we demonstrate any effect of seed size or the interaction of seed size and population on survival or final leaf area or dry weight of the total plant. Nevertheless, we conclude for several reasons that native plants are better adapted to the "home" habitat than alien plants. Firstly, at the shore meadow at Kwade Hoek more seedlings from the native population than from population Angeren survived during the first season and the leaf biomass of native plants was higher (possibly coupled with a lower susceptibility to grazing by slugs or a higher salt tolerance). Secondly, in the greenhouse experiment the reduction of dry weight of leaves of lines from Kwade Hoek, due to grasses, was lower than that of lines from Angeren in the greenhouse experiment, and therefore lines from the shore meadow have a higher competitive ability (sensu Fenner 1978). The greather competitive ability of plants from the shore meadow was probably caused by a higher investment of biomass in photosynthetic tissue. Thirdly, a higher proportion of the lines from population Angeren flowered in the greenhouse on day 50 after sowing compared to lines from population Kwade Hoek. These differences in precocity could not be demonstrated in the present reciprocal sowing experiment. However, in a reciprocal transplant experiment, in which the growing season was longer, more plants from population Angeren flowered at the river bank than from

population Kwade Hoek (Lotz and Blom 1986, unpublished results).

Lotz and Blom (1986) suggested that a high degree of phenotypic plasticity in pattern of biomass distribution over shoot and roots may be an adaptive response to selective forces related to interspecific competition. By changing the shoot-root ratio a plant may adjust its ability to compete either for light or for nutrients and thus consolidate its pre-reproductive development. In the present experiment, however, no differences between plants from the "low competitive" environment Angeren and the "high competitive" Kwade Hoek in level of plasticity in LWR could be detected (the interaction grass density x population was not significant). The higher plant biomass and reproduction of Kwade Hoek lines in the greenhouse experiment was coupled with a higher LWR (and also a higher leaf angle) over all grass densities, than Angeren lines. A high LWR and leaf angle will cause a relatively high light interception by *P. major* ssp. *pleiosperma* in a gap created by cattle.

Although no effects of seed size on final leaf area or plant dry weight could be demonstrated, significant negative effects of seed size on RGR of the shoot during an early part of the reciprocal transplant experiment and percentages of flowering were present. Several authors (e.g. Wulff 1973, Fenner 1983, Zimmerman and Weis 1983) reported that seedlings from smaller seeds had higher growth rates during the early stage of development than those from larger seeds. At the same limited level of nutrient supply small seedlings from small seeds might experience less growth reduction per unit plant weight than relatively large seedlings from large seeds. In addition, large seeds may posses lower concentrations of nutrients (Fenner 1883), which fact might increase the differences in RGR between seedlings from small and large seeds. Moreover, the RGR of *P. major* seedlings tends to decrease as the plant size increases (Dijkstra and Lambers 1986). This reduction of RGR is probably related with changing patterns of allocation. Therefore, the developmental state of the seedlings might have also influenced the present results on RGR. Seed size also affected the chance to flower. Plants that emerged from relatively large seeds had a lower percentage of flowering in the reciprocal sowing experiment. In the greenhouse experiment the negative effect of seed size on percentage of flowering could only be demonstrated for plants from population Kwade Hoek. As yet no physiological mechanism is known to explain the effect of seed size on flowering.

Evolutionary consequences of seed size in P. major ssp. pleiosperma

In the present experiments seed size did not positively influence seedling establishment and growth until reproduction. The effect of seed size on

competitive ability of seedlings might be only present at specific high levels of plant-plant interference (Fenner 1978, Gross 1984, Stanton 1984, Wulff 1986a and b, Kromer and Gross 1987) and these levels of competition are obviously not reached in the (experimental) gaps. Furthermore, the results suggest that plants that emerge from relatively large seeds have a lower probability to flower than plants that emerge from relatively small seeds. Therefore, it is concluded that in these habitats relatively large seeds of *P. major* ssp. *pleiosperma* have probably no adaptive significance for the emerging seedling.

This conclusion seems to be in conflict with the observation that there are more large seeds in the shore-meadow population -an environment with a high level of competition- than in the river-bank population. The relatively large seeds of the shore meadow might be the result of selection in the past when seedlings possibly experienced higher levels of plant-plant interference. However, it is perhaps more likely that selection on seed size of *P. major* ssp. *pleiosperma* from the point of view of the parent plant may have more evolutionary significance than of the seedling that emerged from the seed. If at the shore meadow a short period spent as seed has indeed adaptive value, this fact might influence the trade-off between the number and the size of seeds a plant can produce. Namely, a short seed phase of the offspring may, at least partly, be realized by producing relatively large seeds. Therefore, it is hypothesized that a cost - in terms of fitness - of producing small seeds in the shore-meadow population is more related to the germination pattern of seeds produced by the mother plant than to the competitve ability of the seedling. To test this hypothesis a cost-benefit analysis needs to be applied to seed number and seed size, using long term demographic data on germination and life time reproductive succes.

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CHAPTER 4

THE RELATION BETWEEN AGE AND SIZE AT FIRST FLOWERING AND RELATIVE FITNESS OF PLANTAGO MAJOR L. IN VARIOUS HABITATS

L.A.P. Lotz

Summary. To investigate whether differences in flowering phenology can be explained by different selection regimes, survival, age and size at first flowering and seed production of plants from five populations of *Plantago major* were studied in a reciprocal transplant experiment. Survival until first flowering differed markedly between the study sites. Survival in the pre-reproductive phase was relatively low on a river bank, a beach plain and a path, compared to that in a shore meadow and a dune grassland. Age at first flowering depended on the site as well as on the population from which plants originated. Plants from the river bank, the beach plain and the dune grassland flowered, on average, in an earlier year than plants from the other populations. Selection differentials of age and size at first flowering were determined for the reproductive phase. Three different selection regimes were demonstrated, in which plants had the highest fitness if they 1. flowered early and were large, 2. flowered early and were small, or 3. flowered late and were large. At the path, early reproduction was negatively related to future reproductive output. At this trampled site, plants that initiated flowering relatively early had a lower chance to reproduce a second time than plants that flowered only in a later year.

It is concluded that the between-population differences in age and size at first flowering corresponded generally with the different selection regimes before and after initiation of flowering. Differences between populations in the plastic responses in these life-history characteristics are discussed in relation to habitat characteristics and costs of early reproduction.

Age and size at first reproduction are thought to be primary life-history traits under selection (Samson and Werk 1986, Stearns and Koella 1986). Various authors have related genetic variation in timing of reproduction to selection regimes experienced by plants, e.g. for annual weeds related to different regimes of weed control, low nutrient availability, or short growing seasons (Sørensen 1954); for biennials related to the occurrence in their geographic range (Lacey 1986, De Jong and Klinkhamer 1987), for grassland species related to the time of haying (Ter Borg 1972, Van Groenendael 1985, Van der Toorn et al. submitted) and for annual herbs related to herbivory in specific periods of the growing season (Schemske 1984). Early reproduction may increase relative fitness of genotypes because in this way they can avoid a high risk of mortality before reproductive maturity or bad growth conditions during late reproduction. However, early reproduction or increased investment in reproduction early in the life cycle may cause a reduced life span of the parent and a reduction of future reproductive output and is therefore expected to induce a "cost" in life-history evolution (e.g. Law et al. 1977, Reznick 1985). Costs of early reproduction may not only represent a trade-off within the individual plant -e.g. biomass allocated to seeds is not invested in stems and leaves-, but may also be expressed through interaction between an individual plant and environmental factors. In such cases, studies in the natural habitat will provide a more comprehensive assessment of these costs than studies in the laboratory (Reznick 1985).

Lotz and Blom (chapter 2) and Lotz and Coops (chapter 3) demonstrated genetic differences in time of initiation of flowering between populations of *Plantago major* L. ssp. *pleiosperma* Pilger in greenhouse experiments. Plants from a river-bank population flowered earlier than plants from a population in a shore meadow. Lotz and Blom (1986) demonstrated similar differences in a reciprocal transplant experiment that lasted only one growing season. In the present paper the relationship between time of first flowering and relative fitness of plants from five populations of *P. major* is studied in a reciprocal transplant experiment over three years. The following questions will be addressed here:

1. Do the five populations differ in age and size of plants at first flowering?
2. Can these differences in age and size be explained by the different selection regimes?
3. How do costs, if any, of early reproduction become manifest in the various habitats?

Results of reciprocal transplant experiments only reflect survival and

performances of plants from a specific stage of the life history, i.e. the established seedling, and may therefore limit the scope of the conclusions. As a check, data on survival rates and flowering in the transplant experiment were compared with results of demography of naturally occurring *P. major* in the various habitats.

MATERIALS AND METHODS

The species and the study sites

Plantago major L. is a self-compatible, wind pollinated perennial species with a high rate of self-fertilization (Van Dijk and Van Delden 1981). Two subspecies have been distinguished (Mølgaard 1976, Van Dijk 1985): subspecies *pleiosperma* Pilger and subspecies *major*. The former subspecies is characterized by a high winter mortality in its natural habitat. Within the latter subspecies two ecotypes have been described (Van Dijk 1984): the road-side type, which is trampling resistant (Blom 1979), and the lawn type (adapted to mowing and grazing). *P. major* is an iteropareous species that in winter time loses its above-ground parts.

The populations investigated (three of subspecies *pleiosperma* and two of subspecies *major*) are located in the Netherlands (Table 1). They were selected because of their marked differences in habitat characteristics such as nutrient levels and coverage of higher plants. Lotz and Blom (1986) have described the habitats of the three populations of subspecies *pleiosperma* in more detail. The habitat of the river-bank Angeren (ANG) is characterized by a high temporal variability due to unpredictable floodings in winter, spring and autumn. In summer the site is moderately grazed by cattle. Compared to the other sites the soil is quite rich in total phosphate. Cover by higher plant is relatively low. The Oostvoornse Meer (OM) site is a former beach plain, embanked in 1965. The soil is low in total nitrogen and total phospate. The area is flooded after heavy rain and during winter. Higher plant cover is relatively low. The site is grazed by rabbits. The Kwade Hoek (KH) site is a shore meadow, extensively grazed by cattle. The soil is rich in organic matter and total nitrogen. The vegetation is dense and highly productive. The Weevers' Duin (WV) population is located on a path with a compact sandy soil. In summer plants may suffer from unpredictable severe drought. The site is grazed by rabbits. The last site, Westduinen (WD), is an old coastal grassland, used as pasture for cattle and horses. The soil is intermediate to high in total nitrogen, total

Table 1. Some habitat characteristics of investigated populations of Plantago major. A. subspecies major and B. subspecies pleiosperma. The values of soil characteristics and standing crop are means computed over mean values of July 1984, 1985 and 1986 (S.E. between brackets).

Population		Environment	Soil (0-0.15 m)			Vegetation	
			Total N (mg/100g)	Total P (mg/100g)	Organic matter (%)	Standing crop (g/m^2)	Higher plant coverage (%)
A. Angeren	(ANG)	river bank	62 (14)	95 (8)	1.8 (0.2)	5.6 (0.7)	1 - 10
Oostvoornse Meer	(OM)	beach plain	33 (1)	15 (1)	0.9 (0.0)	5.6 (2.2)	25 - 30
Kwade Hoek	(KH)	shore meadow	504 (47)	41 (5)	13.2 (1.5)	30.6 (6.0)	80 - 99
B. Weevers' Duin	(WV)	path	116 (11)[1.]	33 (1)[1.]	2.8 (0.2)[1.]	12.8 (3.8)	25 - 45
Westduinen	(WD)	dune grassland	334 (45)	30 (3)	8.3 (0.4)	9.2 (1.3)	85 - 92

[1.] Analyses refer to the soil fraction <2 mm (c. 60 per cent of the total sample).

phosphate and organic matter, compared to the other sites. Coverage of higher plants is relatively high. Wolff (1988) demonstrated morphological (determined in the greenhouse) and electrophoretic variability between the five selected populations.

Bulk samples of mature seeds from each population were collected in October 1983. The number of mother plants was 30 at the OM site and 10 at the other sites. The seeds were stored for further use at 4 °C in the dark.

Demography of naturally occurring plants

Demography of naturally occurring seedlings of *P. major* was studied in permanently marked plots from April 1984 to October 1986 (at each site 4 - 10 plots, plot size 0.25 x 0.50 m^2) using colour photographs (method after Blom 1987). Survival and flowering of individual plants were monthly recorded from April to October. Ages at first flowering were estimated only roughly due to the relatively long intervals between countings.

Reciprocal transplant experiment

On 28 May 1984 seeds from each bulk sample were sown in containers with moist dune sand that were placed in a greenhouse (20° C). At least 95% of the various seed collections germinated within three days, but for seeds from ANG this percentage was 60%. When the seedlings were about 16 days old, they were exposed to outdoor conditions for several days before being transplanted. The plants were randomly selected and transplanted into the five study sites during the period 18-22 June 1984. Naturally occurring *P. major* plants were found all-over each transplant-site. At each study site 72 seedlings from each population were planted alternately in a grid (completely randomized design within each site). The shortest distance between plants was 0.10 m. The seedlings were transplanted into small holes with a minimum of disturbance of the surrounding vegetation. The average shoot dry weight of the seedlings (n=10) at the time of transplanting was for the population ANG 1.0 mg (S.D. 0.5), OM 1.4 mg (S.D. 0.7), KH 2.1 mg (S.D. 0.5), WV 1.6 mg (S.D. 0.7) and WD 2.4 (S.D. 0.8). One week after transplanting mortality of the transplants was very low (c. 1%). On this day dead seedlings were replaced.

Survival and flowering were recorded for individual plants during a monthly census until October 1984, and from April to October 1985 and 1986. Leaf area of flowering plants was estimated by multiplying the number of leaves (N) by the length (L) and the width (W) of the largest leaf. This product is also highly correlated with the dry weight of the

leaves of this species (Lotz and Blom 1986). In September and October mature spikes were harvested and the number of seeds per plant was determined.

Data analysis

Analyses of frequencies of categorical variables, including age expressed in month or years, were performed using tests of independence based on the log-likelyhood ratio statistic G (Sokal and Rolhf 1981); unplanned comparisons between subsets were tested by the simultaneous test procedure after Sokal and Rohlf (1981). Leaf area, as an indication of shoot size, was analysed by analysis of variance after log-transformation. For this characteristic unplanned comparisons of means were made by performing the Tukey's honestly significant difference method. In testing differences in numbers of seeds per plant only plants were included that had initiated flowering. Because of departures from normality (many flowering plants did not produce mature seeds) these differences were tested per site by Kruskal-Wallis analysis of variance. The frequency of second flowering and the number of seeds produced at that time were analysed only for plants that had initiated flowering in 1984 or 1985.

Simple and multiple regressions were performed, using relative components of fitness to quantify directional selection on plant age and size at first flowering within one generation (cf. Lande and Arnold 1983, Arnold and Wade 1984, Endler 1986). Selection differentials (covariances between relative fitness and plant characteristics) were computed. These measure the change in the mean value of the characteristic during the selection episode and can be partitioned into a component due to the direct contribution of this characteristic to the measure of relative fitness and a set of indirect contributions due to correlations between this specific characteristic and other plant characteristics (Mitchell-Olds and Shaw 1987, Van Tienderen submitted). The fitness components that were analysed were the total number of seeds produced by plants within the three years the experiment lasted (only flowering plants included), and the number of seeds produced at the first reproduction, the survival until the second flowering and the number of seeds produced at that second reproduction (only plants included that initiated flowering in 1984 or 1985).

RESULTS

Demography of naturally occurring plants

Survival until first flowering was relatively low at all study sites (Table 2). The highest percentage of survival until flowering was found at the beach plain OM, and the lowest in the shore meadow KH (differences among all populations: $G=20.6$, d.f.=4, $P<0.001$). In contrast to the other sites, at the river bank ANG, flooded in winter, no plants survived until the next year.

All reproductive plants at ANG and 8% of those at OM flowered within the year they emerged. At the other sites plants flowered only in the second or third year (Table 2). Only at ANG and WV some plants already started to flower in July; at the other sites flowering started in August (Table 2).

Only at the beach plain OM a few monitored plants survived until the second flowering (Table 2). Obviously, the plot size per site used for studying demography of the naturally occurring seedlings was too small to analyse differences in probabilities of flowering more than once.

Reciprocal transplant experiment

A. Survival and flowering in the reciprocal transplant experiment

Plant survival appeared to depend greatly on the location of transplantation (Table 3.). At the sites KH and WD which have a relatively dense vegetation survival of plants in autumn 1984 was lower than at the other sites (over all sites: $G=333.3$, d.f.=4, $P<0.001$; contrasts between subsets $P<0.001$). At the KH site more plants from the own population survived until that time than plants from populations ANG and WD ($P<0.05$). At the WD site plants from the populations WD and OM had a higher survival rate than plants from the other populations ($P<0.05$). At the river bank ANG, none of the transplants survived the winter flooding of 1984-1985 and at KH only a few survivors of the KH population were found in the spring of 1985 (data not shown). At the latter site, too, no plants survived until the autumn of 1985. At the sites OM, WV and WD various plants were still alive in the autumn of 1986. At site OM no significant differences in survival rate between populations were found at that time, whereas at the WV site plants from population WV and WD had a higher survival than the other populations ($P<0.05$). In the dune grassland WD mortality in 1986 was relatively high compared to that at the OM and WV sites. At site WD plants from the own population and from population WV

Table 2. Summary of results of demography of naturally occurring Plantago major at five study sites during the period 1984 - 1986. Survival rate until first flowering was computed over plants that emerged in 1984 or 1985 and survival rate until second flowering over plants that flowered at least once.

Study site	Number of seedlings per year (m^{-2})	Survival until first flowering (%)	Distribution of age at first flowering (number of plants) 1st	2nd	3rd year	Months in which flowering starts	Survival until second flowering (%)
ANG (river bank)	49	10.8	17	0	0	July - August	0
OM (beach plain)	140	22.9	5	32	28	August - September	3
KH (shore meadow)	43	3.9	0	0	2	September	0
WV (path)	90	19.3	0	23	9	July - September	0
WD (dune grassland)	78	14.5	0	7	1	August - September	0

Table 3 Survival and reproduction in a reciprocal transplant experiment that started at 28 May 1984 with Plantago major plants from five populations The number of survivors in autumn are given (initial number 72) The percentage of surviving plants that produced seeds is indicated between brackets

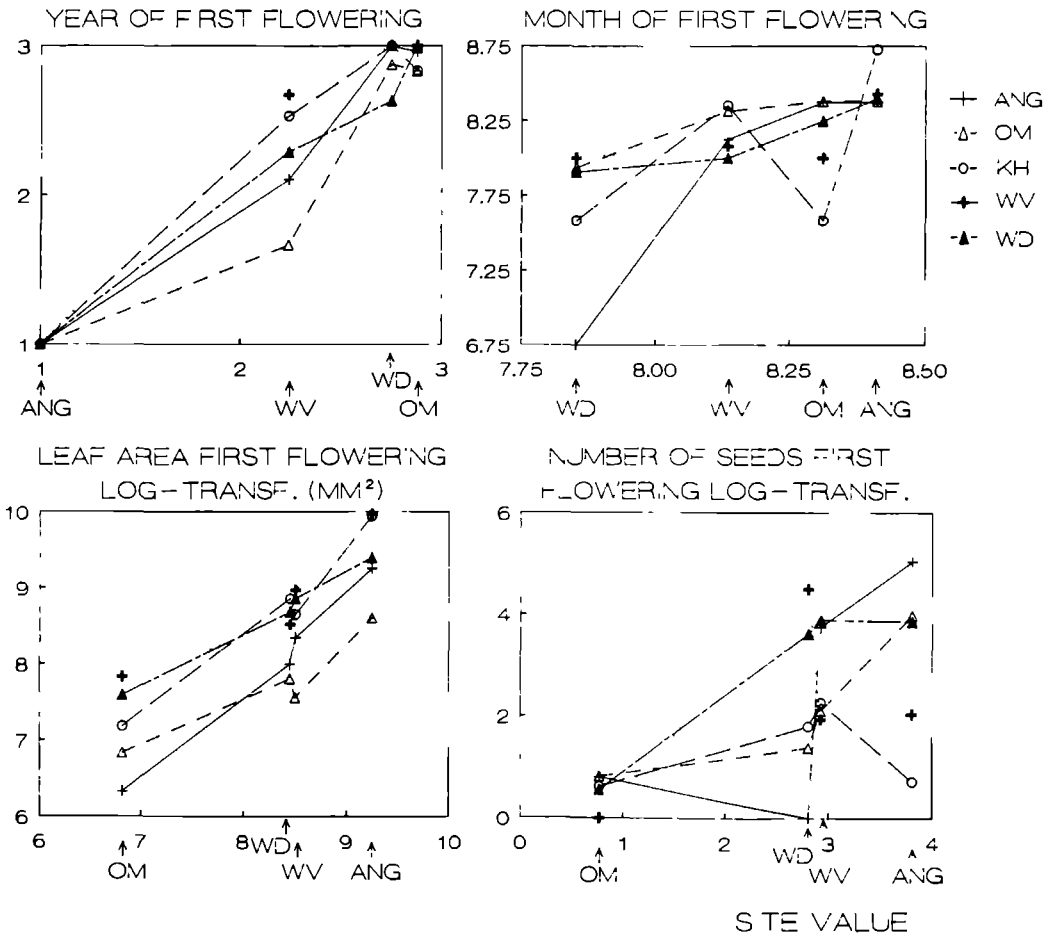
Site	Population	1984	1985	1986
ANG (riverbank)	ANG	54 (70 4)	0	0
	OM	56 (62 5)	0	0
	KH	57 (8 8)	0	0
	VV	45 (17 8)	0	0
	VD	64 (45 3)	0	0
OM (beach plain)	ANG	62 (0)	62 (1 6)	49 (22 4)
	OM	64 (0)	64 (10 9)	52 (75 0)
	KH	61 (0)	57 (1 8)	49 (4 1)
	VV	59 (0)	53 (0)	49 (0)
	VD	59 (0)	59 (0)	43 (2 3)
KH (shore meadow)	ANG	5 (0)	0	0
	OM	16 (0)	0	0
	KH	24 (0)	0	0
	VV	11 (0)	0	0
	VD	6 (0)	0	0
VV (path)	ANG	63 (0)	49 (40 8)	47 (59 6)
	OM	53 (0)	45 (37 8)	34 (41 2)
	KH	61 (0)	47 (6 4)	32 (18 8)
	VV	66 (0)	64 (12 5)	63 (25 4)
	VD	64 (0)	61 (52 5)	60 (68 3)
VD (dune grassland)	ANG	13 (0)	1 (0)	1 (0)
	OM	33 (0)	7 (0)	7 (28 5)
	KH	12 (0)	6 (0)	5 (20 0)
	VV	15 (0)	16 (0)	9 (11 1)
	VD	36 (0)	25 (20 0)	23 (52 2)

had a higher survival than the other plants in the autumn of 1986 ($P<0.05$).

Only at the river bank ANG transplants flowered the first year (Table 3). At the other sites plants initiated flowering in the second or the third year of the experiment. The age at first flowering differed between sites and populations of origin. At WV the largest variation in mean year of first flowering was found, ranging from 1.7 year for plants from population OM to 2.7 year for plants from the own population (Fig. 1A, between populations: $G=88.6$, d.f.=8, $P<0.001$). At the other sites most plants started to flower in the same year. In addition, the timing of the flowering within the season also differed between sites and populations (Fig. 1B, $G=58.3$, d.f.=36, $P<0.05$). Plants from population ANG and KH on average flowered earlier within the season than plants from the other populations. Plants from OM flowered relatively late in the season, compared to the mean values of the other populations at the same sites. The estimated leaf area at first flowering ranged from 909 mm^2 at OM to 10370 mm^2 at ANG (site effect: F:47.7, d.f.=3,51, $P<0.001$). This characteristic also showed a significant site x population interaction ($F=23.9$, d.f.=12,511, $P<0.01$). Taken over all sites plants from the populations ANG and OM had on average a lower leaf area at the start of flowering than plants from the other populations (each contrast $P<0.05$). At each site the latter plants had values for this characteristic that were equal or greater than the mean at the specific sites, whereas those of plants from ANG and OM tended to be lower. The results suggest that plants from population ANG have a higher level of phenotypic plasticity in leaf area at first flowering over the range of the four sites than plants from population OM (Fig. 1C). However, the slopes of the curves (computed from the linear components of the between-site sum of squares) in the environmental sensitivity plots were not significantly different ($P>0.05$).

At the ANG site in the autumn of 1984 more surviving plants from the own population and from the populations OM and WD produced seeds than those from the populations WV and KH (Table 3, contrast between subsets: $P<0.001$). At OM also more surviving plants from the own population reproduced than those from the alien populations ($P<0.001$). At WV at that time the percentage of plants from the populations WD and ANG that reproduced was higher than in case of the populations WV and KH ($P<0.01$). Only at the sites ANG and WV differences between populations in numbers of seeds per plant at first reproduction were significant (only plants included that initiated flowering; Kruskal-Wallis one-way analysis of variance: $G=25.8$, d.f.=4, $P<0.001$ and $G=25.8$, d.f.=4, $P<0.001$, respectively). At ANG the highest number of seeds were produced by plants from the own population and the lowest by plants from KH (Fig. 1D). At WV the highest number of seeds at first reproduction was produced by plants from ANG and WD, and, remarkably, the lowest by

Fig. 1. Environmental sensitivity plots (Falconer 1981) for age and size at first flowering and seed production in a reciprocal transplant experiment with plants from five populations of Plantago major. The mean value of each population at a specific site is plotted against the mean value of all flowering plants at that site. ANG (river bank), OM (beach plain), KH (salt meadow), WV (path) and WD (dune grassland). Year and month are expressed as rank numbers (1984=1 and January=1, respectively).



plants from WV. At WD no differences between populations in frequencies of reproductive plants and seed production could be demonstrated, probably due to the lower number of surviving plants.

Due to the restriction that second flowering could only be studied on plants that initiated flowering in 1984 or 1985, the number of plants involved in that analysis was relatively low (Table 4). At WV a higher proportion of plants from population WD flowered twice compared to plants from populations WV and OM ($P<0.05$). With respect to the total seed production per plant over three years the same trends in differences between populations were found as in the number of seeds at first reproduction; however differences between populations at site WV were even larger (data not shown).

Table 4 Percentage of Plantago major plants that flowered a second time in a reciprocal transplant experiment, expressed as percentage of the plants that produced seeds in the first two years Populations ANG (river bank), OM (beach plain), KH (shore meadow), WV (path) and WD (dune grassland)

Site	Population	Number of reproductive plants in 1984 or 1985	% second flowering
OM	ANG	1	100
	OM	9	33
	KH	1	0
WV	ANG	38	32
	OM	39	10
	KH	8	0
	WV	15	13
	WD	42	55
WD	OM	1	100
	WD	7	29

B. Characterization of selection regimes

At site ANG the correlation between age at first flowering and seed production, during the short flowering season, was significantly negative, while the correlation between seed production and the leaf area at that time was significantly positive (Table 5A). The differences in the selection differentials and their direct components were small, thus these trends in selection were hardly influenced by correlation between timing of flowering and plant size.

At site OM the total number of seeds per plant was negatively correlated with the year of first flowering, but positively correlated with the month in which flowering was initiated (Table 5A). In addition, seed production was negatively correlated with the leaf area at first flowering. The selection differential of 0.5 month for this within-season age was mainly determined by the indirect contribution of the year of first flowering -being 0.3 month-, due to a negative correlation between month and year at first flowering (not shown), while the direct contribution of the month of first flowering was only 0.1 month. The selection differential of -0.9 mm^2 of leaf area, too, was mainly made up of the indirect contribution of the year of first flowering -being -0.4 mm^2-, due to a positively correlation between leaf area and year of first flowering, and the direct contribution of the leaf area, being -0.4 mm^2. Thus, at this beach plain plants were selected that flowered late in the season and had a relatively small shoot, but flowered in a relatively early year.

Site WV was the only environment in this study where a considerable number of plants flowered twice. Therefore, only at this site components of fitness associated with repeated flowering could be analysed. No significant correlations between the number of seeds per plant at first reproduction and age and leaf area at this first flowering could be demonstrated. However, for year and leaf area at first flowering direct components of the selection differentials with significant partial correlation coefficients existed (Table 5A). At WV, plants that survived until second flowering began to flower, on average, later and had a larger shoot at that time than plants that died or didnot flower anymore after their first reproduction (Table 5B). Selection for age and size at first flowering could not be demonstrated with respect to the fitness component number of seeds at second reproduction (Table 5B). However, the aforementioned correlation between leaf area at first flowering and frequency of second flowering was associated with a significant positive correlation between leaf area at first reproduction and the total number of seeds produced per plant at the path during the experiment (Table 5A).

At WD the total seed production per plant was negatively correlated with the year of first flowering and positively correlated with leaf area at that

Table 5 Characterization of selection on age (in years and in months within one season) and leaf area of Plantago major at first flowering Plants were grown in a reciprocal transplant experiment at sites ANG (river bank), OM (beach plain), WV (path), WD (dune grassland) Selection differentials (S), the component of S due to direct selection (S_d), with the multiple r^2, and the corresponding levels of significance of the (partial) correlation coefficients are given Components of fitness $Seeds_1$, the number of seeds produced at the first reproductive stage, $Flow_2$, survival until the second stage of flowering, $Seeds_2$, the number of seeds produced at the second stage of flowering, $Seeds_{tot}$, the total number of seeds produced
ns not significant, * P<0 05, ** P<0 01, *** P<0 001, - no analysis performed

Plant characteristic at first flowering A	Site ANG n=191		Site OM n=90	
	$Seeds_{tot}$		$Seeds_{tot}$	
	S	S_d (r^2= 25)	S	S_d (r^2= 18)
Age -(year)	-	-	- 4***	- 3**
-(month)	- 2***	- 3***	5*	1ns
Leaf area (mm^2)[1]	4**	6***	- 9*	- 4ns

	Site WV n=218				Site WD n=32	
	$Seeds_1$		$Seeds_{tot}$		$Seeds_{tot}$	
	S	S_d (r^2= 04)	S	S_d (r^2= 08)	S	S_d (r^2= 26)
Age -(year)	- 0ns	- 2*	- 1ns	- 3***	- 2*	- 3ns
-(month)	- 2ns	- 3ns	- 2ns	- 3*	2ns	- 1ns
Leaf area (mm^2)[1]	3ns	3*	3**	4**	3*	3*

B. Site W

	$Flow_2$ n=142		$Seeds_{23}$ n=41	
	S	S_d (r^2=.09)	S	S_d (r^2=.06)
Age -(year)	.1*	.0ns	.1ns	.0ns
-(month)	-.2ns	-.2ns	-.2ns	-.2ns
Leaf area (mm^2)[1].	.4***	.4**	.2ns	.2ns

[1]. Regression analysis after log-transformation.

time (Table 5A). At this dune grassland differences between the selection differentials of these traits and their direct components were small.

DISCUSSION

As suggested by Charlesworth and Leon (1976) and Law et al. (1977) survival rates in the pre-reproductive period might affect the optimal (in fitness terms) age at reproductive maturity. These authors state that genotypes that experienced about the same risk of mortality over a wide range of ages were selected for having a greater reproductive effort early in their life cycle, whereas genotypes that suffered from high mortality early in life (thus having a concave survivorship-curve) were selected for having a greater reproductive effort later in the life cycle, e.g. through iteroparity. Survival rates of *P. major* until flowering at the five study sites will be discussed first. Subsequently, differences between populations will be related to this pre-reproductive survival and to selection regimes determined in the reproductive phase. Finally, some aspects of costs of early reproduction and local adaptation will be discussed in more detail.

Survival rates in the pre-reproductive period

Survival until flowering was relatively high at the river bank ANG -but at this site no plants survived the floodings during the subsequent winter-, at the beach plain OM and at the path WV. Apparently, the relatively low concentrations of N and P in the soil of OM do not affect survival of *P. major*. At the shore meadow KH and the dune grassland WD survival rates until flowering were low. At these pasture-like sites mortality of seedlings was high, probably due to high levels of interspecific competition (Lotz and Blom 1986, Lotz and Coops submitted). The latter authors demonstrated that in a sowing experiment in artificial gaps at the shore meadow, survival until flowering was considerably higher than in the present experiment. The numbers of plants involved in this study, were obviously too small to analyse reproduction of transplants at these two sites. According to Charlesworth and Leon (1976) and Law et al. (1977), one may expect on account of pre-reproductive survival rates, that at ANG, OM and WV genotypes will be selected that flower early in the life cyle, whereas at KH and WD selection for later flowering may occur.

At all sites trends in survival and flowering were about the same in the demographic study of naturally occurring plants as in the reciprocal transplant experiment.

Age and size at first flowering: genetic variation and selection regimes

At OM and WD most plants flowered only in the third year, probably due to low availability of nutrients and high level of intraspecific competition, respectively. At these sites no genetic differences between populations in age of first flowering could be demonstrated. At ANG flowering in the second year could not be studied. However, the results of the first year demonstrate that at this site plants from ANG, OM and WD flowered, on average, in an earlier year than plants from KH and WV (had the latter plants have had the opportunity). At the path WV, plants from population OM flowered, on average, in an earlier year than plants from the own population.

At ANG relative fitness of plants was favoured by flowering early in the season and at OM and WD by flowering in an early year. Neither of these trends in selection could be demonstrated at KH and WV. At KH very few plants will probably reach the reproductive phase and might then produce many seeds, e.g. through iteroparity (cf. Blom 1987). At WV the chance to flower a second time, and thus to increase total seed production, was higher for plants that had a relatively large shoot at first flowering. This plant size could probably not be reached while flowering in an early (i.e.

the first) year. In contrast, at ANG plants were selected that flowered early after germination, while having a relatively large shoot. Such a life-history strategy might only be realized in environments where relative growth rates are high (Stearns and Koella 1986). Relative growth rates of *P. major* determined at ANG were indeed high compared to those at OM and KH (Lotz and Blom 1986). At the beach plain OM, with a soil poor in nutrients, plants increase their seed production by initiation of flowering in an early year, which trend may be realized by flowering relatively late in the season while being still relatively small.

The age at first flowering of plants from the populations of subspecies *pleiosperma*, ANG and OM (both flowering relatively early in life) and KH (flowering relatively late), correspond with values one may expect to be the result of both the selection regime in the pre-reproductive phase, as suggested by Charlesworth and Leon (1976) and Law et al. (1977), and the selection in the reproductive period, as quantified in this study. For both populations of subspecies *major* the relation between genetic variation in these life-history characteristics and selection regimes seems to be more complicated. Although at WV the pre-reproductive survival was relatively high, the plants from this path population initiated flowering relatively late. However, according the quantified selection in the reproductive phase, reproduction late in the life cycle enhances iteroparity, and therefore fitness at this trampled site. The relatively early reproduction of WD plants corresponds, too, with the selection regime in the reproductive phase, in spite of the relatively low survival rates in the pre-reproductive phase.

Costs of having the potential of early reproduction in P. major

The present results show that plastic responses in age and size at first flowering differed between the investigated populations. Although Lotz and Blom (1986) demonstrated that levels of phenotypic plasticity for various life-history characteristics were high, e.g. compared to the variation due to genetic variability, plants from KH and WV were practically not able to shorten their pre-reproductive periods at the river bank. Such genetically based differences in plastic responses might simply represent different morphogenetic constraints (Watson and Casper 1984). However, a cost of early flowering, in terms of fitness, is a more likely explanation for these differences between populations of one species. Costs of reproduction are usually difficult to quantify (Reznick 1985) and might be only apparent under controlled conditions (Primack and Antonovics 1982). In this study I demonstrated that at the path WV, plants that initiated flowering relatively early and that had a lower shoot size at that

time, had a lower chance to reach a second reproduction. However, for a detailed characterization of the costs associated with early initiation of first flowering in *P. major*, a study over many years is required in which allocation patterns to reproductive tissues are related to life-time reproductive succes.

Local adaptation

Although the main interest in this study is focussed on flowering phenology in relation to fitness, some remarks on local adaptation, shown during the period of three years after transplantation, are worthwhile. Various authors reported genetic differentiation between populations in specific characteristics, that might be explained by local adaptation (e.g. Venable 1984, references cited in Introduction). The present results suggest that local adaptation at least occurred at ANG and OM. At these sites plants from the own population had both a higher survival and a higher proportion of flowering than plants from several other populations. At the path WV during the experiment plants from the own population and from population WD had a greater survival than plants from the other populations. Plants from WV and WD, both belonging to subspecies *major*, are probably better adapted to soil compaction, trampling and summer drought (Blom 1979). This higher tolerance might be related to the lower specific leaf area of plants from subspecies *major* compared to plants from subspecies *pleiosperma* (Dijkstra in press). However, within the observation period the native transplants had a lower proportion of flowering and a lower seed production at WV than plants from OM and WD.

Antonovics and Primack (1982) discussed several reasons for the phenomenon that alien genotypes have a higher fitness at a specific environment than native genotypes. One explanation might be that site characteristics are not representative during the experiment (e.g. specific favourable conditions in fluctuating environmental factors are lacking). An other explanation might be that not the whole life history of the plants was studied and that therefore the results were biased (Watkinson and Gibson 1985, Van Groenendael 1985). A three-years experiment may probably be to short to study flowering and life-time reproductive succes in the path environment.

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CHAPTER 5

VARIATION IN LIFE-HISTORY TRAITS WITHIN A SUBDIVIDED POPULATION OF PLANTAGO MAJOR L. SSP. PLEIOSPERMA PILGER

1. TEMPORAL AND SPATIAL VARIABILITY OF SOIL PROPERTIES IN A FORMER BEACH PLAIN

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Summary. As part of a research project on the variation in life-history characteristics within a population of *Plantago major* L. ssp. *pleiosperma*, seasonal and spatial variability in the availability of macronutrients (N, P, and K) was examined on a small scale in the 0-25 cm soil depth at a primary beach plain site, embanked since 1966. On the basis of distinct differences among other things in plant biomass, an *a priori* division into three different types of microhabitat occurring in a mosaic distribution pattern has been made: an overall low-lying area (subsite 1) with slightly elevated patches of 0.5 to 1.5 m in diameter (subsite 2) and rather large patches, 20 to 40 m in diameter, of sea buckthorn shrubs, with small and relatively open spots (subsite 3) in the transitional zone from lower area into shrub. All three subsite types were studied within one area of approximately 2000 m^2 with a proportional ratio of 12 : 1 : 7 for subsites 1, 2, and 3, respectively.

Three methods of analysis were applied: an inventory survey (sampling once at the start of the growing season), an analysis of the seasonal variation (sampling at approximately monthly intervals during the period April - November), and an assessment of nitrogen mineralization potentials in the laboratory (sampling once at the beginning of the growing season). All three procedures clearly demonstrated the occurrence of differences in the availability of nutrients over very short distances, i.e. a pronounced spatial variability among subsites.

This small-scale differentiation in soil properties has occurred in an essentially homogeneous parent material (e.g. in texture and carbonate content) over a period of about 20 years. Particularly the availability of N and P appeared to have increased at the small elevations and in the sea

buckthorn shrub (subsites 2 and 3, respectively), when compared to the low-lying patches (subsite 1) of the beach plain area. Besides a spatial variability, statistically significant temporal fluctuations were observed in the availability of N, P, and K. Relative fluctuations of mineral N (as indicated by the range/mean ratio) were especially large at the small elevations and in the sea buckthorn shrub.

Key words: soil nutrient availability - soil - nutrient pools - spatial variation - temporal variation

INTRODUCTION

The influence of time and space should be considered in any study of mineral nutrition in relation to plant distribution (Gupta and Rorison 1975). Even in a natural soil, at first sight uniform over distances of meters, the range of available habitats in terms of soil properties can be large and is in itself as ecologically significant as are the mean characteristics of the site (Ball and Williams 1968). Each study at the level of plant populations will, therefore, require generally an investigation of the spatial and temporal patterns of environmental factors. However, information on soil variability and its possible ecological or evolutionary implications is rather limited (Davy and Taylor 1974, Gupta and Rorison 1975, Snaydon 1970, Taylor et al. 1982, Veresoglou and Fitter 1984). Moreover, the smaller the scale of interest, the more difficult it is to infer useful common generalizations from reported case studies. The complexity of a dynamic soil environment is another factor that often contributes to the problem of extrapolating information from one soil or site to another (Peterson and Hammer 1986). Detailed soil studies (including data on mineralization potentials and seasonal variability) in relation to the process of genetic differentiation within plant populations have to the authors knowledge not yet been published. In one study, population differentiation has been demonstrated over distances of less than 30 m in *Anthoxanthum odoratum* within 40 years, in response to a mosaic of environmental heterogeneity (Snaydon 1970).

In their study on phenotypic plasticity in life-history traits of *Plantago major* ssp. *pleiosperma*, Lotz and Blom (chapter 2) selected three populations which differed markedly in habitat characteristics (geographically different locations in the Netherlands). They demonstrated the existence of genetic variation among these populations, in levels of

plasticity in biomass allocation to vegetative and generative parts of the plant, in response to differences in nutrient supply. The present study is part of a research project on the variation in life-history characteristics of *P. major* ssp. *pleiosperma* on a much smaller scale (order of 10 m). For this purpose three subpopulations were distinguished within one of the former three populations (population 'Oostvoornse Meer', chapter 2). The subpopulations occurred in clearly identifiable microhabitats in a small-scale mosaic environment of a former beach plain, embanked about twenty years ago. Plants of these subpopulations differed markedly in several life-history properties, such as biomass, flowering time, and reproductive output (Lotz et al. chapter 6). The occurrence of obviously larger individuals at two of the three microhabitats without any dilution effect in macronutrient concentrations (Table 1) suggested a much higher nutrient availability at these two subsites.

The main objective of the research project was to investigate whether within this beach plain population differences in life-history characteristics and in plasticity in these properties could have evolved as an adaptation to patterns of abiotic environmental factors fluctuating in time and/or space. The present study is the first in a series and aims at

Table 1. Mean shoot weights and concentrations of N, P, and K in shoots of Plantago major ssp. pleiosperma sampled in July at three selected subsites at location Oostvoornse Meer (40 plants were sampled per subsite)

Subsite	Shoot dry weight	Organic N	K	H_2PO_4
	$mg.plant^{-1}$	$mmol.kg^{-1}$	$meq\ kg^{-1}$	$meq.kg^{-1}$
		(shoot dry weight)		
1	10	870	497	34
2	78	917	725	49
3	71	882	676	42

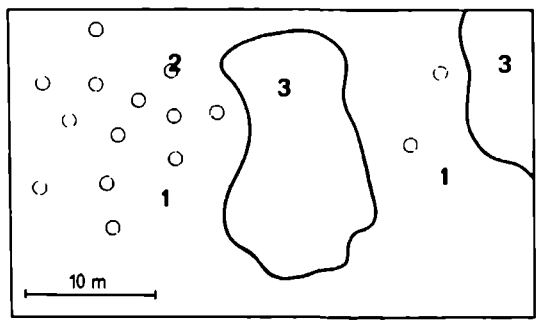
quantifying the seasonal and spatial variability of a number of mainly soil chemical factors within an approximately 2000 m^2 site at location Oostvoornse Meer. All three subpopulations of *P. major* ssp. *pleiosperma* that were distinguished were present in this study area in their corresponding microhabitats. The principal objective of the present paper was to determine the variation among these microhabitats in the availability of nutrients and in nutrient pools in the 0-25 cm soil depth, the zone of maximum root activity for *P. major* ssp. *pleiosperma* in this area. The mosaic pattern of soil properties must have developed since the embankment, thus within the last 20 years. Selection differentials of inbred lines, originating from the three identified subpopulations, were studied in a reciprocal transplant experiment in the study area (chapter 6), whereas responses to specific ranges of abiotic factors in life-history traits of these inbred lines were investigated in the greenhouse (chapter 7 and 8).

MATERIALS AND METHODS

Site description

The study site Oostvoornse Meer is a former beach plain on the southwest coast of the Netherlands, embanked in 1966, and has since developed into a primary dune slack. Because of rising of the groundwater table, relatively large depressions in the area often become waterlogged in winter and early spring. The stage of soil development in this fundamentally homogeneous parent material is very young and accordingly, the calcareous sand substrate is relatively low in total nitrogen. The slightly higher parts of the area are 20 to 40 m in diameter and are colonized by dense and relatively young shrubs of sea buckthorn (*Hippophae rhamnoides* L. ssp. *rhamnoides*), a species capable of fixing dinitrogen in combination with actinomycetes of the genus Frankia. The vegetation of the open low-lying parts is rich in species. However, higher plant cover is low here (30%) and plants, mostly short-lived perennials, are characterized by both a low growth rate and a low total biomass. Within this overall lower part of the area, several small elevations (approximately 0.1 m high and 0.5 to 1.5 m in diameter) are present, which are characterized by a higher plant cover of especially grasses, a clearly increased plant biomass production (as compared to the surrounding lower area), and frequent visits by rabbits (relatively high amounts of droppings). The main grazing pressure in the area is from these rabbits. Plants of *Plantago major* L. ssp. *pleiosperma* (Pilger) grow in all

Fig. 1. Schematic representation showing the relative distribution of the three subsites at location Oostvoornse Meer; 1= overall low-lying area, 2= small elevation, and 3= Hippophae shrub



three subsites, i.e. the overall lower part, the small elevations, and the small open spots in the transitional zone into a *Hippophae* shrub (further referred to as subsites 1, 2, and 3, respectively; Fig. 1).

Climate

Total precipitation amounted to about 870 mm during the year of investigation, which is considerably higher than the normal annual figure of 725 mm for this area. About 65 per cent of the total rainfall occurred in the periods June-August (330 mm) and November-December (230 mm). The period of investigation (April-November) followed a for this area rather severe winter (mean temperatures for January and February -2.1 and -0.9 °C, respectively).

Layout of the sampling area and sampling techniques

Within an area of approximately 45x45 m^2, six locations of each subsite were selected randomly and their position marked. Per subsite, three of these locations were chosen for an inventory survey of soil properties, whereas the remaining three served for the study of seasonal fluctuations.

For the inventory analysis, a 45x45 cm^2 grid subdivided into nine units of 15x15 cm^2 was used for each location. On 1 May 1985 nine soil cores (3 cm diameter) were taken per location, one from each grid unit. As the

greater part of the roots of *P. major* ssp. *pleiosperma* were present within a maximum depth of 25 cm, this layer was sampled in four increments: 0-5, 5-10, 10-15, and 15-25 cm. The nine samples were subsequently bulked per location and soil depth. After drying (35°C) and sieving (2 mm), bulk samples were mechanically subdivided and part of the sample was ground in a mortar mill. Analyses were performed on either ground or unground samples, as indicated below.

For the study of seasonal variation, the same 45x45 cm^2 grid was used and on each occasion laid out in the same position. Sampling took place at approximately monthly intervals during the period 16 April - 4 November. Per sampling date and location, intact soil cores (1.3 cm diameter) were taken from one randomly selected grid unit (excluding previously used units). Three depths were sampled: 0-5, 5-15, and 15-25 cm. Samples were transported in an insulated container to the laboratory and freshly analyzed without any further pretreatment. All analyses were completed within two days after sampling.

In addition, to obtain an impression of the net nitrogen mineralization potential, 0-10 cm soil cores (3 cm diameter) were taken on 1 May 1985 from the same three locations per subsite as used for the inventory study: two cores per grid unit (18 per location, 54 per subsite). The cores were left intact, put in polypropylene centrifuge tubes, transported to the laboratory in an insulated container, and incubated aerobically at 30°C and at field moisture content (present at sampling) for six periods varying from 4 to 20 weeks. Also from the same grid, nine cores (1 per grid unit) were taken per location for the estimation of initial moisture contents. On each occasion, three cores per location and nine cores per subsite were extracted and analyzed.

Soil analyses

Results are expressed on a dry weight basis. Where appropriate, bulk density estimates for the respective depths were used to express parameters in terms of a standard volume or as amounts in the soil profile per m^2.

Inventory study. The pH of the soil was measured potentiometrically in 1:2.5 (w/v) suspensions of unground soil in H_2O. Carbonates were measured gas-volumetrically by treating ground soil with 4 *N* HCl. Organic matter was estimated as loss-on-ignition, i.e. weight loss of ground samples after ignition at 430°C for 24 h (Davies 1974). Total P and total N were determined colorimetrically following digestion of ground samples with a mixture of H_2SO_4-Se and salicylic acid (Novozamsky et al. 1984). Olsen P and labile organic P were measured in a 0.5 *M* NaHCO3 extract of unground soil (w/v 1:20; Bowman and Cole 1978b, Olsen et al. 1954,

Watanabe and Olsen 1965). Exchangeable K, Na, and Mg were determined by atomic absorption spectrophotometry after shaking unground soil with neutral ammonium acetate (w/v 1:25). Chloride and electrical conductivity analyses were carried out on 1:5 (w/v) water extracts of unground soil. The granular composition (soil texture) was determined by sieving.

Study of seasonal variation. Soil properties measured were moisture content, pH-H_2O, NH_4-N, NO_3-N, 'available' P (Olsen P and labile organic P), exchangeable cations (K, Na, Mg), chloride and electrical conductivity. Soil moisture (drying for 24 h at 105°C) was determined in duplicate on two separate soil cores. The (fresh)soil:solution ratios applied in the various extraction procedures were 1:2.5 (pH), 1:5 (chloride and electrical conductivity), and 1:10 (exchangeable cations, bicarbonate P, mineral N). Ammonium and nitrate were determined in 1 *N* KCl extracts by steam distillation using MgO and Devarda alloy (Keeney and Nelson 1982). Ammonium was determined in the distillate by the indophenol-blue method (Kempers 1974).

Nitrogen mineralization potential. Following incubation in the laboratory, the intact soil cores were crumbled and the complete amount of soil extracted with 1 *N* KCl (soil:solution ratio 1:5). Ammonium and nitrate were determined by steam distillation as in the seasonal study, but the ammonium in the distillate was measured either by titration with 0.01 *N* potassium bi-iodate (high concentrations) or by the indophenol-blue method (low concentrations, Kempers 1974).

Statistical analyses

Soil layers originating from the same cores are dependent on each other and data were, therefore, always analyzed per depth increment. In most cases, log transformations were applied to data sets to establish homogeneity of variance.

In the inventory study a one-way analysis of variance (ANOVA) was performed, whereas data of the seasonal study and of the incubation experiment were analyzed according to a split-plot-in-time design with subsites as main plots. Tukey's test was used to identify significant differences among means.

Table 2. Mean soil characteristics of three selected sub sites at location Oostvoornse Meer, as determined in the inventory survey (sampling in triplicate per subsite) and in the seasonal study (8 days during the period 16 April - 4 November; sampling in triplicate per subsite and date). Significant differences among subsites ($P<0.05$; Tukey's HSD test) are indicated per layer (column)

Soil property	Subsite	Inventory survey				Seasonal study		
		Depth (cm)						
		0-5	5-10	10-15	15-25	0-5	5-15	15-25
Organic matter (%)	1	2.4 a	0.34 a	0.20 a	0.12 a			
	2	5.7 b	0.71 b	0.47 b	0.18 b	-	-	-
	3	2.5 a	0.43 a	0.29 a	0.20 b			
$CaCO_3$ (%)	1	6.2 b	6.7	6.2	6.3			
	2	4.7 a	6.1	6.9	6.7	-	-	-
	3	5.4 ab	6.5	6.1	5.9			
pH-H_2O	1	7.8 c	8.5 b	8.6 b	8.7 b	7.8	8.4	8.6
	2	7.1 a	8.1 a	8.4 a	8.6 ab	7.7	8.4	8.6
	3	7.5 b	8.3 ab	8.4 a	8.5 a	7.7	8.5	8.7
Moisture (%)	1					41.6	24.7 ab	25.2
	2	-	-	-	-	46.9	26.4 b	25.3
	3					31.7	17.2 a	20.1
Total N (ppm by volume)	1	1099 a	230 a	163	65			
	2	1933 b	419 b	240	96	-	-	-
	3	1164 a	210 a	167	120			
NH_4 N (ppm by volume)	1					1.7 a	0.8	0.6
	2	-	-	-	-	2.9 ab	1.8	0.8
	3					3.9 b	1.3	0.8

NO_3-N (ppm by volume)	1					1.1	0.7	0.6 a
	2	-	-	-	-	1.4	1.1	0.8 a
	3					2.8	1.1	1.4 b
Soil solution NO_3^- concentration (mM)	1					0.15 a	0.13 a	0.11 a
	2	-	-	-	-	0.24 ab	0.20 ab	0.15 a
	3					0.62 b	0.40 b	0.34 b
Total P (ppm by volume)	1	249 ab	243 b	237 b	214			
	2	267 b	189 a	237 b	226	-	-	-
	3	212 a	170 a	190 a	184			
Olsen P (ppm by volume)	1	6.2 a	2.1	2.4	2.8	1.7 a	0.8	1.0
	2	19.8 b	2.9	2.8	2.7	3.6 ab	1.4	1.4
	3	6.4 a	1.7	1.5	1.8	4.9 b	1.1	1.1
Labile organic P (ppm by volume)	1	7.2 a	1.1 a	0.4	0.2 a	1.7	0.2 a	0.18 a
	2	14.5 b	3.2 b	1.6	0.6 ab	3.6	0.8 b	0.21 ab
	3	7.1 a	1.0 a	1.0	0.9 b	3.2	0.5 ab	0.5 b
Exchangeable K (meq.L^{-1} of soil)	1	2.0 ab	1.3	1.3	1.2	2.5	1.2	1.4
	2	3.8 b	1.2	1.2	1.0	3.1	1.7	1.8
	3	1.1 a	0.8	0.9	0.7	2.5	0.7	0.4

RESULTS AND DISCUSSION

Inventory survey

The results of the inventory samples, taken at the beginning of the growing season, are summarized in Table 2. Differences among subsites were greatest in the upper layers and much less pronounced or absent in the subsoil. The soils have a fine sandy texture (70-85 per cent in the range 106-212 um) with only very minor differences in the upper layers of the subsites (not shown). Organic matter and nutrient concentrations were as expected highest in the upper 5 cm and relatively high at subsite 2. Some differences appeared to be close to significance ($0.05 < P < 0.10$): exchangeable K (5-10 cm), Olsen P (10-15 cm), and total N and total P (15-25 cm). Soil reaction and carbonate content were significantly lower in the upper layer of subsite 2. Subsite 3 was relatively low in total P, compared to the other two sites, but this was also the site with the highest aboveground standing crop (*Hippophae* shrub).

Since very little and often not statistically significant variation was observed among subsites in chloride, sodium, magnesium, and electrical conductivity, these properties have been left out of consideration, here and in the following.

Study of seasonal variation. Table 2 summarizes the overall seasonal means, whereas seasonal variations are presented in Fig. 2. The results for NO_3-N are also given as average concentrations in the soil solution (calculated as $100a/14b$ m*M* NO_3^-, where a= ppm NO_3-N and b= % moisture, both on a dry weight basis). The main effect of subsite as a variance source was significant at the 0.05 level in about one-third of the ANOVA tests (Tables 2 and 3), whereas some differences in macronutrient concentrations were close to significance ($0.05 < P < 0.10$): NO_3-N (0-5 cm), NH_4-N and Olsen P (5-15 cm), and exchangeable K (15-25 cm). The main effect of date was significant in 16 out of 24 tests, and mostly at the 0.001 level (Table 3). Significant subsite x date interactions occurred in 8 cases, although only once in the upper 5-cm depth. Significant effects of date and subsite x date interaction were referring especially to factors related to differences in water regime at the subsites (e.g. sodium and chloride; not shown in Table 3); macronutrients N, P, and K were much less involved, particularly in the upper 15 cm of the soil profile.

All subsites appeared to have a relatively high moisture content throughout the season and on each sampling date moisture contents in the upper layers generally followed the order subsite 3 < subsite 1 < subsite 2, the relatively high position of subsite 2 being caused mainly by its higher

Fig. 2. Fluctuations in moisture, pH, mineral N, bicarbonate extractable P, and exchangeable K at different depths in the soil profile at three selected subsites at location Oostvoornse Meer

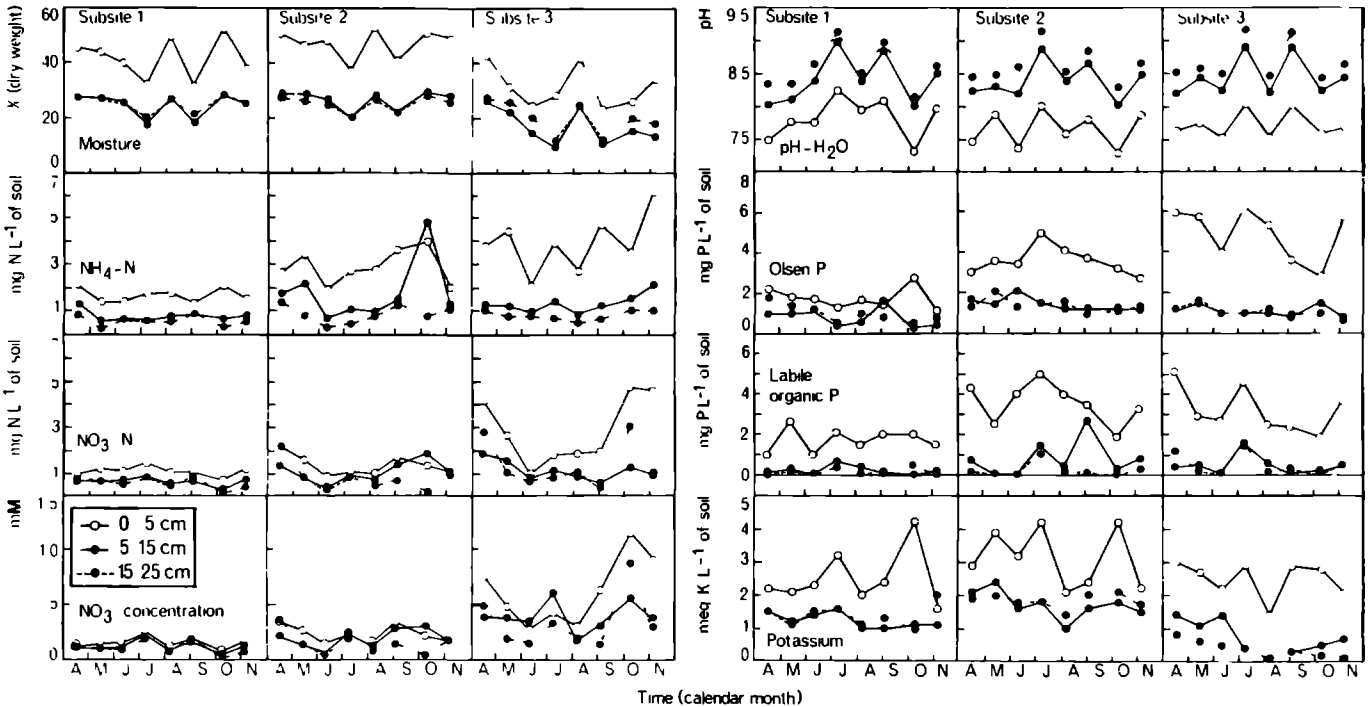


Table 3. F values and significance of variance sources of eight soil properties at three depths at location Oostvoornse Meer (seasonal study). The effect of subsite was tested against the error mean square of R (replication) + SxR with df = 6; effects of date and subsite x date interaction were tested against the error mean square of DxR + SxDxR with df = 42. Levels of significance: ns not significant, * P<0.05, ** P<0.01, *** P<0.001

Variance source	df	Moisture	pH	NH_4-N	NO_3-N	Solution NO_3	Olsen P	Labile organic P	K
0-5 cm depth									
Subsite (S)	2	3.24 ns	1.55 ns	6.42 *	3.92 ns	5.24 *	5.51 *	3.14 ns	1.20 ns
Date (D)	7	11.9 ***	25.6 ***	1.96 ns	1.98 ns	2.23 ns	0.96 ns	2.34 *	2.94 *
SxD	14	1.92 ns	2.96 **	1.32 ns	1.30 ns	1.40 ns	1.30 ns	1.28 ns	0.61 ns
5-15 cm depth									
S	2	7.50 *	0.23 ns	4.78 ns	3.22 ns	5.30 *	3.74 ns	5.88 *	3.19 ns
D	7	55.3 ***	50.7 ***	1.30 ns	1.18 ns	2.98 *	2.00 ns	3.71 **	2.10 ns
SxD	14	5.86 ***	2.44 *	0.42 ns	1.06 ns	0.99 ns	0.87 ns	2.12 *	0.56 ns
15 25 cm depth									
S	2	4.27 ns	0.47 ns	3.25 ns	9.76 *	8.75 *	1.09 ns	6.21 *	4.60 ns
D	7	46.5 ***	54.9 ***	3.22 **	7.82 ***	8.45 ***	4.52 ***	3.97 **	2.78 *
SxD	14	5.02 ***	1.62 ns	1.95 ns	4.44 ***	4.23 ***	1.20 ns	1.31 ns	2.10 *

organic matter content (Table 2). The excessive rainfall in the period June-August (330 mm of the annual total of 870 mm) is expressed at all sites and in all layers in a clearly increased moisture content in August (Fig. 2). Around the second last sampling date, two of the three replicate locations at subsite 1 became temporarily waterlogged. Subsites 2 and 3 should compare favourably with subsite 1, especially at the start and the end of the growing season, in terms of physical properties such as soil aeration and thermal regime, with its impact on biological processes such as root and microbial activity.

Average pH values of the different subsites were essentially the same (Table 2) with only minor differences in fluctuations (Fig. 2). When working with fresh soils, one would expect the pH to decrease on drying, partly as a result of varying (dry)soil: solution ratios. However, since the pH of alkaline soils will have a tendency to change to the neutral point after flooding (Patrick and Mikkelsen 1971), lower pH values during the winter are to be expected, especially at subsite 1. Indeed, relatively low pH values were recorded at all sites during spring and late autumn (Fig. 2); the correlation between pH and moisture in the 0-5 cm depth was significant and negative (Table 4) and increased in significance for the deeper layers: simple correlation coefficients (r) of -0.58 and -0.67 for the 5-15 cm and 15-25 cm depths, respectively.

Nutrient concentrations were generally highest in the upper soil layers (Table 2, Fig. 2). Instant nutrient availabilities are the resultant of differences between inputs (e.g. mineralization) and outputs (e.g. plant uptake, leaching). The declining K levels during the growing season, particularly at subsites 2 and 3, have probably been influenced by plant uptake as commonly observed (Haines and Cleveland 1981, Peterson and Rolfe 1982, Vaughn et al. 1986, Veresoglou and Fitter 1984), whereas leaching may have contributed to the relatively low K levels generally found in August. However, availability of K was not expected to be a growth limiting factor at these subsites, as indicated also by the relatively high concentrations of K in the plant material (Table 1).

Where fluctuations in mineral N were most pronounced (subsites 2 and 3; Fig. 2), relatively low levels were observed in June, which is most likely due to increased growth rates and nutrient absorption by plants as reported by many workers (Davy and Taylor 1974, Gupta and Rorison 1975, McGarity and Myers 1973, Peterson and Rolfe 1985, Taylor et al. 1982, Vaughn et al. 1986, Williams 1969). Later in the season, during the fall-off in the growth of plants, rates of mineralization apparently exceeded rates of uptake, resulting in concentrations of mineral N increasing again (Fig. 2). The quantity of NH_4-N almost invariably exceeded that of NO_3-N in these calcareous soils, a phenomenon observed also by Davy and Taylor (1974) and Taylor et al. (1982) for chalk soils. Although low in comparison

with the normal range in fertile agricultural soils of 2 to 20 m*M* (Mengel and Kirkby 1987), soil solution NO_3^- concentrations were clearly higher at subsite 3 (*Hippophae* shrub) with > 0.3 m*M*, compared to subsites 1 and 2 with <0.3 m*M*. It should be noted that, irrespective of differences in average plant size, organic N concentrations in plants from the different subsites were of a similar low level (Table 1).

In contrast to soil nitrogen, factors affecting the availability of soil phosphorus can be far more complex due to the presence of different inorganic and organic phases. These different P forms are subject to many interactions and physico-chemical and microbial processes, which are affected by e.g. moisture, temperature, and pH. This rather complex behaviour of soil P is probably the main reason that soil P properties sometimes showed more irregular or less pronounced temporal patterns of variation than soil N, or were less consistent with an annual pattern of plant P uptake (Friesen et al. 1985, Harrison 1979, Saunders and Metson 1971, Vaughn et al. 1986). In the present study, available inorganic P concentrations in the upper 5 cm showed a decline at all subsites for the greater part of the season (Fig. 2). On the other hand, a gradual increase or relatively high levels were observed during the period April-July at subsites 2 and 3, respectively. These latter observations agree to some extent with the concept that available P may build up concurrently with increases in plant growth during spring-early summer (Harrison 1979, Saunders and Metson 1971). Although the subsequent decreases in available P at subsites 2 and 3 might be associated with a relatively increased plant uptake, patterns of available P and mineral N did not exactly coincide in this respect (Fig. 2), whereas Gupta and Rorison (1975) found qualitatively similar patterns of variation in the amounts of available nitrogen and phosphorus.

It is generally agreed that the role of organic P is of major importance in the phosphorus cycle in natural vegetations in the temperate regions (Cole et al. 1977, Halm et al. 1972) and total bicarbonate extractable P (Olsen P + labile organic P) is likely to be a better measure of plant available P than inorganic P (Olsen P) only (Abbott 1978, Bowman and Cole 1978a). Olsen P and labile organic P showed roughly similar patterns of fluctuation (Fig. 2) and were, therefore, highly correlated in the upper layer (Table 4). On the one hand, a significant positive correlation between Olsen P and labile organic P is conceivable from the fact that both parameters are determined in the same extract; moreover, it has been pointed out (Abbott 1978) that the Olsen P fraction may include some easily hydrolysable organic P that is mineralized during the period of extraction. On the other hand, if it is assumed that cycling of organic P is of major importance for the within-season utilization of P by plants growing in these relatively young calcareous soils low in P, positive correlations between labile organic P and

Table 4. Correlation matrix (r-values) among several measured soil variables in the 0-5 cm layer (seasonal study, complete data set, n = 68; dry weight basis where relevant). Levels of significance: ns not significant; all tabulated values are significant at the 0.01 level, except for values in parentheses, which are significant at the 0.05 level

	1	2	3	4	5	6
1 pH	-					
2 Moisture	-0.37	-				
3 K	ns	(0.30)	-			
4 NO_3-N	ns	ns	ns	-		
5 NH_4-N	ns	ns	0.40	0.61	-	
6 Olsen P	ns	ns	0.47	(0.26)	0.53	-
7 Labile organic P	ns	0.33	0.51	ns	0.39	0.81

available inorganic P are rational. Also, the positive correlation between NH_4-N and bicarbonate extractable P (Table 4) might be suggestive of a rather close relationship between P availability and mineralization processes.

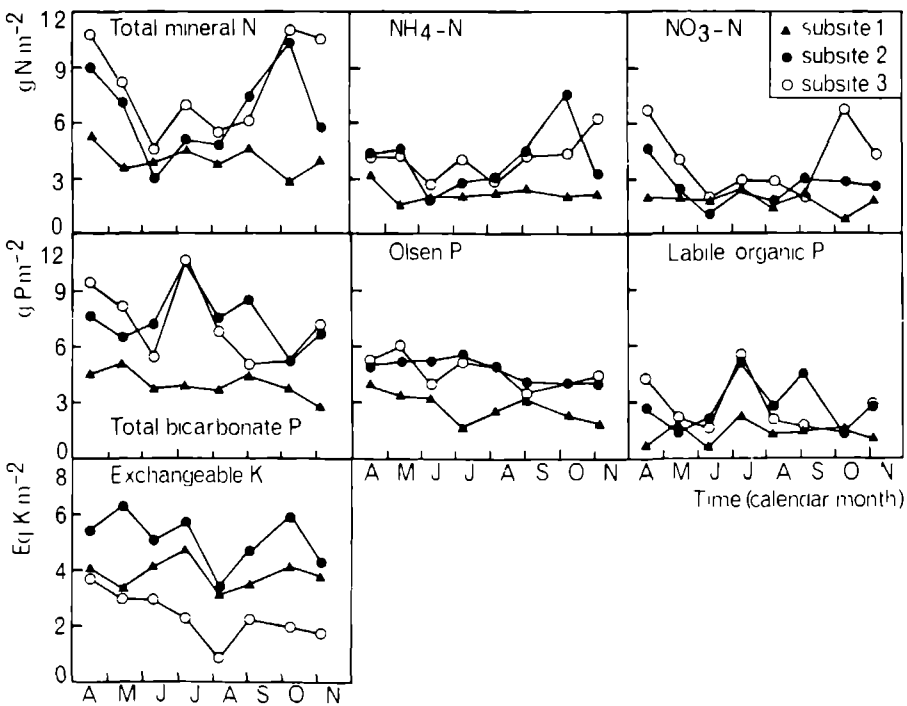
The low biomass production in the overall low-lying area (subsite 1) is probably caused primarily by a very short supply of P, although preliminary fertilization experiments in the same area also indicated a limitation by N (R. Baas, unpublished results). The P concentration in plants from subsite 1 (Table 1) comes very close to reported minimum levels for *Plantago* species: 20 to 30 meq.kg^{-1} shoot dry weight (De Jager and Posno 1979, Troelstra et al. 1983). Kachi and Hirose (1983) concluded that plants growing in coastal calcareous sand dune soils were deficient in nitrogen, rather than phosphorus. However, concentrations of P in their control test plants were about twice as high as in the present study, the nitrogen concentrations being essentially similar.

When comparing the inventory and seasonal analyses (Table 2), differences in e.g. Olsen P and labile organic P are probably largely due to pretreatment effects.

Nutrient pools

Since the analyses of the three successive layers (coming from the same soil cores) cannot be considered to be independent and the combining together of layers might accentuate differences among the subsites, a separate analysis of the complete 0-25 cm depth was performed. Mean element pools were calculated for both the inventory and the seasonal study (Table 5). Except for labile organic P and exchangeable K in the seasonal study, all other factors were significantly different among subsites, some effects

Fig 3. Fluctuations in macronutrient pools (mineral N, bicarbonate extractable P, exchangeable K) in the 0-25 cm depth at three selected subsites at location Oostvoornse Meer



of subsite being even significant at the 0.01 level: total N, total P, Olsen P, labile organic P, and total bicarbonate P. Significant effects of date occurred in NO_3-N ($P<0.01$), total mineral N ($P<0.05$), labile organic P ($P<0.001$), and exchangeable K ($P<0.01$). Except for labile organic P ($P<0.05$), all subsite x date interactions were not significant.

Seasonal fluctuations of nutrient pools in the 0-25 cm depth (Fig. 3) demonstrated even better some of the conclusions reached in the previous section. The relatively low amounts of total N, total P, and exchangeable K at subsite 3 (Table 5, Fig. 3) can be related in part to the high aboveground standing crop of the *Hippophae* shrub. Estimating a standing crop of 2.5 kg dry matter per m^2 (F.C. Zoon, pers. comm.), and N, P, and K concentrations of about 1600 $mmol.kg^{-1}$, 50 $meq.kg^{-1}$, and 200 $meq.kg^{-1}$ dry matter, respectively (Troelstra et al. 1987), this corresponds with about 56 g $N.m^{-2}$, 4 g $P.m^{-2}$, and 0.5 Eq $K.m^{-2}$.

As to the availability of the macronutrients N and P, the curves in Fig. 3 and the seasonal data in Table 5 clearly reveal a division of the three subsites in favour of the small elevations and the *Hippophae* shrub (subsites 2 and 3). The range/mean ratio as determined in the seasonal study (Table 5) can be considered as a relative measure of the seasonal variation (Peterson and Hammer 1986). Relative fluctuations of mineral N were particularly large at subsites 2 and 3.

Nitrogen mineralization potential

Upon incubation of intact soil cores in the laboratory, NO_3-N appeared to be the only form of mineral N accumulating in these calcareous soils (Fig. 4). Largest flushes of NO_3-N were observed for subsites 2 and 3, these being significantly higher than that of subsite 1 (Tukey's HSD test, $P<0.05$). When expressed as percentage of total N, the following trend was found among subsites (Fig. 4): subsite 3 > subsite 2 > subsite 1, with subsite 3 significantly different from subsite 1 (Tukey's HSD test, $P<0.05$). This might be interpreted as being the actual order of turnover rates of the organic N pool occurring at these subsites. Indeed, it often perfectly matched the order of instant mineral N concentrations at the three subsites (Table 2, Figs. 2 and 3).

A significant subsite x time interaction was found (Table 6), which is caused by the terminal leveling off of the curves for subsites 2 and 3 (Fig. 4), probably due to the development of an equilibrium between microbial release and uptake. The production of NO_3-N at subsite 1 continued to increase steadily for the duration of the experiment. Although actual mineralization in the field will differ quantitatively from the estimated potential mineralization in the laboratory, patterns will very likely be

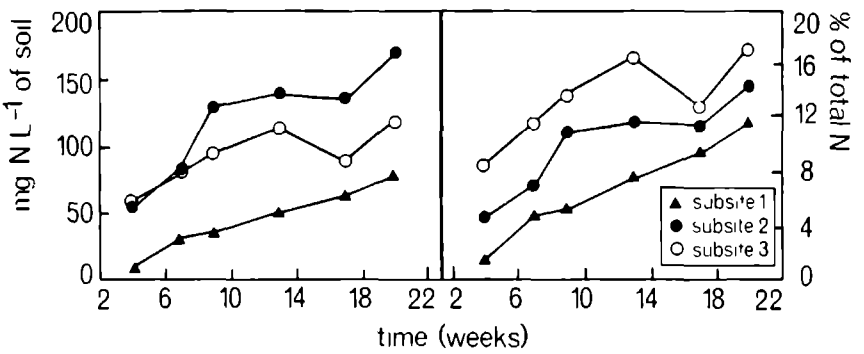
Table 5. Mean element pools ($g.m^{-2}$, unless otherwise indicated) and seasonal variation in the 0-25 cm depth at three selected subsites at location Oostvoornse Meer. Significant differences among subsites are indicated (Tukey's HSD test at the 0.05 level)

Element	Subsite		
	1	2	3
inventory analysis, n=3			
Total N	81 a	139 b	89 a
Total P	58 b	57 b	47 a
Olsen P	0.81 a	1.55 b	0.67 a
Labile organic P	0.45 a	1.03 b	0.54 a
Total bicarbonate P	1.26 a	2.57 b	1.21 a
Exchangeable K ($Eq.m^{-2}$)	0.35 ab	0.41 b	0.21 a

Seasonal study, n=24			
NO_3-N	0.19 a (116)*	0.26 ab (212)	0.39 b (360)
NH_4-N	0.22 a (129)	0.40 b (378)	0.41 b (200)
Total mineral N	0.41 a (93)	0.66 ab (308)	0 80 b (236)
Olsen P	0.27 a (85)	0.47 b (32)	0.46 b (54)
Labile organic P	0.12 (142)	0.28 (136)	0.26 (165)
Total bicarbonate P	0.39 a (62)	0.75 b (72)	0.72 b (75)
Exchangeable K ($Eq.m^{-2}$)	0.40 (43)	0.51 (57)	0.23 (122)

* Range/mean ratio (per cent) in parentheses

Fig 4 Net accumulation of mineral N (NO_3-N) in the 0-10 cm depth at three selected subsites at location Oostvoornse Meer following aerobic incubation of intact soil cores in the laboratory at 30°C Mineral N expressed as mg N L^{-1} of dry soil (left) or as % of total N (right), each point represents the average of 9 determinations



qualitatively similar (e.g. Davy and Taylor 1974).

However, in view of the effects of physical properties (moisture, thermal regime) upon the processes of nitrogen mineralization in the field, the laboratory results for subsite 1 very likely represent a relative overestimation.

CONCLUSIONS

The three methods of analysis used in the present study (inventory survey, analysis of seasonal fluctuation, and nitrogen mineralization potential) clearly demonstrated differences in the availability of nutrients to occur over very short distances in three distinguishable microhabitats in the Oostvoornse Meer area, all colonized by *P. major* ssp. *pleiosperma*. As to the macronutrients N and P, differences in availability among subsites appeared to be both spatially and temporally determined, with particularly large relative mineral N fluctuations occurring at the small elevations (subsite 2) and in the *Hippophae* shrub (subsite 3). The, with respect to most soil properties, relatively optimum position of the subsites 2 and 3 was in agreement with the occurrence of much larger plant individuals at these subsites, as compared to the overall lower area (subsite 1). Because of its position within the *Hippophae* shrub, the shady environment of subsite 3 may represent another potentially important abiotic characteristic in comparison with the open habitats of subsites 1 and 2.

Table 6 F values and significance of variance sources of the net nitrogen mineralization in the 0-10 cm soil layer collected from three selected subsites at location Oostvoornse Meer (incubation experiment) The effect of subsite was tested against the error mean square of R + SxR with df=6, the effect of time against the error mean square of R2 + TxR2 with df=12, and the effect of subsite x time interaction against the error mean square of SxTxR + SxTxRxR2 with df=60 (R = replication within subsites, R2 = replication within R) Levels of significance * P<0 05, ** P<0 01, *** P<0 001

Variance source	df	Net nitrogen mineralization	
		mg N L^{-1} of soil	% of total N
Subsite (S)	2	17 2 **	11 8 **
Period of incubation (T)	5	50 4 ***	35 4 ***
S x T	10	4 75 ***	2 07 *

It should be emphasized that the observed differentiation in soil properties over short distances has taken place in this beach plain area during a relatively short period of soil development (about 20 years) in an essentially homogeneous parent material. The present mosaic structure with only small differences in relief can very likely to a large extent be traced back to patterns of colonisation in the past by perennial grasses (e.g. *Puccinellia maritima*) and sea buckthorn (*Hippophae rhamnoides* ssp. *rhamnoides*), inducing small-scale differences in e.g. sediment, organic matter, and nutrient accumulation. The different levels of nutrient supply among the subsites may have had its consequences for genetic differentiation and/or phenotypic plasticity within the population of *P. major* ssp. *pleiosperma*, in respect to variation in life-history traits.

In addition, when evaluating aspects of soil nutrient availability, the analyses pointed at the possible bias when applying only one procedure (e.g. inventory survey with pretreatment of samples leading to a relative overestimation of subsite 1) or sampling and analyzing fresh soil material only once during the season (e.g. around June with mineral N

concentrations of similar magnitude at all three subsites). Although virtually equal amounts of total N were present in the overall low-lying area and in the *Hippophae* shrub, the results of the incubation experiment nevertheless provided valuable information about distinct differences in nitrogen mineralization potential between these two subsites, indicating the inadequacy of total N measurements.

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CHAPTER 6

VARIATION IN LIFE-HISTORY TRAITS WITHIN A SUBDIVIDED POPULATION OF PLANTAGO MAJOR L. SSP. PLEIOSPERMA PILGER

2. SELECTION AND LOCAL ADAPTATION

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Summary. An attempt is made to relate variation in life-history characteristics within a population of *Plantago major* ssp. *pleiosperma* to small-scale environmental variability. At a primary beach plain, embanked in 1966, a mosaic environment was distinguished with spatial variability in vegetation structure as well as in nutrient availability and water content of the soil. In a reciprocal transplant experiment within this beach plain with lines originating from three subsites, differences in contemporary selection were quantified. Differences in selection regimes existed in direction and strength of selection on specific characteristics of morphology and life history, and in the life-cycle stage in which selection occurred. At a subsite with a low availability of nutrients a relatively high investment in reproductive tissue at first reproduction was correlated with a low probability of survival until the next reproductive period. At the other subsites this reproduction cost could not be demonstrated. From the results of the reciprocal transplant experiment it was concluded that variation in morphology and life-history characteristics was mainly due to phenotypic plasticity. Besides, genetic differences were found between lines from different subsites, which in the case of morphology partly corresponded with the variation in selection regimes. Five reasons are discussed, which might explain a weak correspondence between the demonstrated patterns of contemporary selection and the genetic variation between lines: genetic constraints, genetic drift, changes in the environment due to succession, gene flow (possibly promoted by grazing of mature spikes by rabbits), and finally high levels of phenotypic plasticity in fitness components.

Key words: selection - life-history traits - growth form - genetic

INTRODUCTION

A study of the relationship between variation in plant characteristics within a population and micro-scale heterogeneity of environmental factors offers the opportunity to gain a better understanding of the strategies plants adopt to cope with specific environments. Several authors have pointed out the problems that may arise in testing the adaptiveness of responses of plants to environmental heterogeneity. For example, the environment can vary both in space and time in a complex way and therefore, the relationship between environmental variability and observed variation in life-history traits is difficult to detect (Lacey et al. 1983). Besides, it is difficult to decide which environmental factor's variability is important in terms of fitness of a plant (e.g. Schlichting 1986). Therefore, any study on genetic differentiation and phenotypic plasticity in life-history traits of plants in relation to small-scale environmental heterogeneity should be coupled with detailed studies on environmental variability of the natural habitat and demography of plants occurring in that habitat.

More insight into the relation between variability in life-history characteristics and environmental heterogeneity may be obtained by using two different approaches. Firstly, one may study genetic differentiation in life-history characteristics between subpopulations under various conditions and relate differences in means or in levels of phenotypic plasticity in these characteristics, to variation in environmental factors (e.g. Snaydon 1970, Warwick et al. 1987). Usually, when genetic differences were found in these studies, they were interpreted as being the result of past differences in selection regimes. Secondly, contemporary selection may be studied in natural populations by regression analysis of (components of) relative fitness on plant characteristics. As yet there have been relatively few studies investigating selection in plant populations in this way (Endler 1986). Both approaches are applied in the present study. This study is part of a research project on variability in life-history traits within a beach-plain population of *Plantago major* L. ssp. *pleiosperma* Pilger.

P. major is a self-compatible, wind pollinated perennial herb with a high self-fertilization rate (Van Dijk and Van Delden 1981). In an accompanying paper spatial variation in soil properties at the study site is

described in detail (chapter 5). A mosaic environment can be distinguished with variation in vegetation structure as well as in availability of nutrients and in water content of the soil. Three reasons made this site appropriate for testing the relationship between spatial variability of environmental factors and levels of genetic variation and phenotypic plasticity in life-history traits. In the first place, the characteristics of the sandy soil of this beach-plain have the tendency to vary in space, but to be relatively stable in time (chapter 5). Secondly, in the mosaic environment three types of subsites, including patches of two size classes, (diameter 0.5 - 1.5 m and 15 - 25 m, respectively) can be distinguished. Plants of *P. major* ssp. *pleiosperma* grow and reproduce at each subsite. For that reason, variation in life-history traits can be related to the scale of environmental variability. Thirdly, the former beach plain was embanked in 1966, which implicates that any population structure has become established since then. Therefore, studying differentiation in life-history traits at this beach-plain offers the opportunity to roughly estimate the rates of selection processes in a relatively early stage of a succession series in a coastal environment.

In this paper results are presented from a reciprocal transplant experiment, in which vegetative and reproductive characteristics of lines of *P. major* ssp. *pleiosperma* were investigated. These lines were reared from plants collected at the three subsites. The aimes of this field experiment were: 1. quantifying contemporary selection acting on morphology and life-history characteristics of *P. major* ssp. *pleiosperma* at each subsite; 2. relating differences in selection regimes with genetic variation in morphology, life-history characteristics and fitness components between lines of different origins.

Results of reciprocal transplant experiments only reflect survival and performances of plants for a specific stage of the life history, i.e. the established seedling. As a check, data on survival rates and flowering in the transplant experiment were compared with results of demography of naturally occurring *P. major* L. ssp. *pleiosperma* at the three subsites. At the beach plain at each subsite 10 - 20 per cent of the mature spikes of *P. major* ssp. *pleiosperma* are grazed by rabbits (L.A.P. Lotz and L.D.H. Spoormakers, unpublished results). Germination of seeds of *P. major* ssp. *pleiosperma* from rabbit droppings was studied to test whether seeds that passed through the intestinal canal of rabbits could be neglected as regards the fitness of grazed plants.

MATERIALS AND METHODS

Study site and derivation of lines

The investigated population of *P. major* L. ssp. *pleiosperma* Pilger is located near Oostvoornse Meer, a brackish lake on the south west coast of the Netherlands. The study site, an area of about 2,000 m^2, is a former beach plain, embanked in 1966. The whole area is grazed by rabbits. Due to differences in micro-relief, nutrient availability (for a detailed description see chapter 5), and vegetation structure (Lotz and Olff, 1988) a mosaic environment with three subsites can be distinguished. Subsite 1 (1190 m^2) is a low-lying area; the soil is low in nutrients and water saturated. In winter this area is frequently flooded by rain water. The vegetation is rich in species. Cover by higher plants is relatively low (30%). The standing crop of this open vegetation is c. 15 $g.m^{-2}$. Subsite 2 (total area 110 m^2) consists of small elevations (0.5 to 1.5 m in diameter, 0.07 - 0.15 m high) spread over at subsite 1. The patches are mainly covered with a short, dense, grassy vegetation (standing crop c. 100 $g.m^{-2}$). The soil of these patches is relatively high in nutrients and organic matter. Subsite 3 (total area 700 m^2) consists of relatively large patches (20 - 40 m in diameter, c. 0.15 m high as compared to subsite 1), covered by shrubs of *Hippophae rhamnoides* L. Between these shrubs the standing crop of the herb layer is relatively high (>200 g m^{-2}). At this subsite plants of *P. major* ssp. *pleiosperma* are shaded by the shrubs. Simular to the soil of the patches of subsite 2, the availability of nutrients in the soil is higher and water content is lower when compared to subsite 1.

In autumn 1984 10 reproductive plants, roots partly included, were randomly sampled from each subsite, defoliated and transplanted into pots with potting compost. The plants, which were watered daily, were grown until reproduction in a greenhouse (20°C, light-dark period: 16-8 h). Cross-pollination was prevented, while the plants flowered, by paperbags. Lines were obtained by collecting mature seeds from each plant separately.

Demography of naturally occurring plants and germination from rabbit droppings

Demography of naturally occurring seedlings of *P. major* L. ssp. *pleiosperma* was studied in permanently marked plots from April 1985 to September 1987 (at each subsite three plots, plot size 0.25 x 0.50 m^2). Coordinates were assigned to all germinated seedlings in order to identify each seedling individually. At a monthly census from April to October

(years 1985 and 1986) and from May to September (1987) survival and flowering were recorded for individual plants. In order to obtain a global estimation of the seed production per plant per season, in autumn 1986 the number of seeds produced per plant was determined for ten randomly selected flowering plants per subsite.

On 16 November 1986 at every subsite 300 rabbit droppings were sampled and incubated during 12 days in a cold chamber (4 °C, dark). After this pretreatment, which breaks dormancy of seeds of *P. major* (L.A.P. Lotz, unpublished results), the droppings were spread on moist filter paper in a growth cabinet (22 °C, light regime: 1 W/m^2, light-dark period: 12 h/12 h). Numbers of seedlings emerging from the droppings were recorded during two months.

Reciprocal transplant experiment

On 25 April 1985, seeds of 10 lines from each subsite were sown in containers with moist dune sand that were placed in a greenhouse (20°C). At least 95% of the seeds of each line germinated within three days. On day twenty after sowing the seedlings were exposed for several days to outdoor conditions before being transplanted into the field. Seedlings from each line were randomly selected and transplanted into a randomized complete block design; ten square plots (blocks) were spread over each subsite with a single replicate of each line per plot (total number of plants: 3 x 3 x 10 x 10= 900). Within each plot plants were positioned in a 0.10 x 0.10 m^2 grid. The resulting plant density is within the ranges of naturally occurring adult plants observed at each subsite. The seedlings were transplanted into small holes with a minimum disturbance of the surrounding vegetation. At this time differences in dry weight of the shoots of seedlings of lines from different origins (subsites) were not significant (nested analysis of variance, $F=1.7$, d.f.=2,17, $P=0.21$). One week after transplanting mortality of the transplants was very low (<1%). On this day dead seedlings were replaced. Recordings of survivorship were made in 1985 on 5 June, 29 June, 15 August, 1 October, in 1986 on 21 May, 12 August, 9 September, and 10 October, and in 1987 on 20 May, 10 September, and 12 October. On 1 October 1985 the following characteristics of the surviving plants were determined: the number of leaves (N), the length (L) and the width (W) of the largest leaf (further referred to as leaf length and leaf width, respectively), the length of the lamina of the largest leaf, the number of spikes and the length of the longest spike. Biomass of the leaves was estimated by multiplying N by L by W. This product is highly correlated with the dry weight of the leaves of *P. major* (chapter 2). In addition, the loss of leaf material by grazing

(mainly by rabbits and slugs) was determined and expressed in a ranked variable, coded 0-3: 0 no loss of shoot material, 1 only small holes were eaten in leaves, 2 one or more leaves were partially eaten, 3 one or more laminae of a single plant were totally eaten. In October 1985 and in September and October 1986 (and 1987) mature spikes were harvested and counted. Dry weights of these spikes (including scapes), of the seeds and of 30 randomly selected seeds were determined. Single seed weight was computed from the dry weight of these 30 seeds. The total number of seeds per plant was computed by dividing the total dry weight of the seeds by the single seed weight.

Data analysis

The survivorship curves of naturally occurring and transplanted seedlings were compared between subsites or origins using the Lee-Desu statistic (SPSS-subprogramme SURVIVAL, Nie and Hull 1981). In these analyses data of plants which were alive at the last census date were censored (cf. Pyke and Thompson 1986). Differences in numbers of seedlings that emerged from the incubated droppings, were analysed in a one-way analysis of variance (subsites replicated by six samples of 50 droppings) after square-root transformation.

To analyse selection on transplanted seedlings four components of fitness, that are associated with successive episodes of the life cycle, were distinguished: W1, the survival of seedlings until the first flowering; W2, the number of seeds produced during this first reproductive period; W3, the survival from the first until the second flowering; W4, the number of seeds produced at the second and third periods of flowering.

Techniques for quantifying natural selection within one generation, or 'phenotypic selection', have been discussed by Lande and Arnold (1983), Arnold and Wade (1984), and Endler (1986). In the present study simple and multiple regressions were performed using relative (components of) fitness as dependent variables. The purpose was to characterize directional selection acting on morphological and life-history characteristics in stages of the life cycle that were studied. Selection differentials (the covariances between relative fitness and plant characteristics) measure the change in the mean value of the characteristic during the selection episode. They can be partitioned into a component due to the direct contribution of this characteristic to the measure of relative fitness and a set of indirect contributions, due to correlations between this specific characteristic and other plant characteristics (Mitchell-Olds and Shaw 1987). Such a subdivision in a direct and an indirect part depends on the variables used in the multiple regression. In this study the analysis of selection was

focussed on two different sets of plant characteristics. The first set, which consisted of the number of leaves, the length, width and lamina- leaf ratio of the largest leaf, and the grazing index, all determined in October 1985, was used in regression of the fitness component W1 and the composition W1*W2+W3*W4. Plants used in this analysis were those which had survived at least until October 1985. The second set consisted of characteristics of the first reproductive stage, i.e. the age of the plant, the estimated biomass of the leaves, and dry weights of the spikes, which traits were used in the regression of W3 and W3*W4. In the analyses of the second set only those plants were included which flowered for the first time in 1985 or 1986.

Data on growth, morphology and reproductive outputs were analysed using analysis of variance (SPSS-subprogramme MANOVA, Nie and Hull 1981) with as independent variables: subsite, origin, and line nested within origin. The first two factors are fixed treatment effects, the latter factor is a random effect. Non-significant mean squares were pooled with the within-cells mean squares when appropriate. For the reciprocal transplant experiment differences between plots could not be analysed because of empty cells due to mortality of plants. Analysis of the simple effects of origin within each subsite were made by partition of the pooled sum of squares of the origin effect and of the interaction subsite x origin (*a priori contrasts*). Comparisons of means over subsites were made by performing the Tukey's honestly significant difference method.

RESULTS

Demography of naturally occurring plants and germination from rabbit droppings

Some demographic features of *P. major* ssp. *pleiosperma* at the three subsites are listed in Table 1. In 1985-1987 more seedlings emerged in the plots of subsite 2 than in those of subsite 1 and 3. The median survival time of seedlings was lowest at subsite 3 and highest at subsite 1. Only few plants at each subsite reached the reproductive state. The mean number of seeds per plant was very low at subsite 1, intermediate at subsite 2 and relatively high at subsite 3. The ratio of number of counted seedlings per m^2 and the estimated number of seeds produced per m^2 was low at all subsites, at subsite 1 about 0.19, at subsite 2 0.03, and at subsite 3 0.02. Therefore, from the present demographic results it cannot be concluded that, at any subsite the maintenance of *P. major* ssp. *pleiosperma* depends

Table 1. Summary of results of demography of naturally occurring P. major ssp. pleiosperma at a beach plain. Subsite 1, low-lying area; subsite 2, small patches; subsite 3, large patches with shrubs. Median survival times were computed over all seedlings that emerged within the study period (1985-1987). Number of seeds per plant are means over randomly harvested plants in 1986. Seed production per m^2 was estimated from number of flowering plants per m^2 times number of seeds per plant.

Subsite	Seedlings		Reproduction		
	Number per year (m^{-2})	Median survival time $(month)^{-1}$.	Number of flowering plants per year (m^{-2})	Number of seeds per plant (±S.D., n=10)	Number of seeds x1000 (m^{-2})
1	265	2.8	48	28.4 (± 22.1)	1.4
2	748	1.5	64	384.6 (±321.3)	24.6
3	267	0.7	16	804.1 (±950.1)	12.9

1. Each contrast between subsites significant ($P<0.001$)

on transport of seeds from other subsites.

The number of seedlings of *P. major* ssp. *pleiosperma* which emerged per 50 rabbit droppings was for samples originating from subsite 1, 2 and 3: 3.0, 6.1 and 3.5, respectively (differences between groups were not significant: F=1.5, d.f.=2,15; P=0.23).

Reciprocal transplant experiment

a. Characterization of selection regimes

Directional selection on morphological characteristics of the shoot and on susceptibility to grazing damage was studied using 284 plants of subsite 1, 278 of subsite 2, and 185 of subsite 3 (plants which had survived until October 1985).

For subsite 1 the correlation between the number of leaves in October and the relative fitness component W1 (survival until first flowering), and the partial correlation coefficient of the corresponding multiple regression were significantly positive (Table 2). At this low-lying subsite with a relatively low standing crop, seedlings that flowered at least once had, on average, 0.18 more leaves than the total cohort of seedlings that survived until October 1985. The component of the selection differential due to direct selection was somewhat larger, being 0.3 leaves. Remarkably, the direct component of the selection differential of the leaf length with respect to W1 was negative. Therefore it can be concluded that at subsite 1, during the life cycle until first flowering, plants were selected for having more but shorter leaves (which means that the height of this rosette plant will be low). In contrast, at subsite 2 plants with larger leaves were selected in this part of the life cycle. At subsite 3, although the direct component of the selection differential (with a significant partial regression coefficient) of the leaf length with respect to W1 had a negative value, the regression between W1 and number of leaves was not significant.

Other differences in selection regimes between subsites for the part of the life cycle that was studied, are demonstrated in Table 2. At subsite 1 leaf width has a positive simple correlation with the total seed production (W1*W2+W3*W4). However, significant positive partial correlation coefficients with this fitness component were found only for the number of leaves and the grazing index. In contrast to this, at subsite 2 and 3 positive simple correlations between W1*W2+W3*W4 and the number of leaves and the leaf width were found, and, only at subsite 2, between W1*W2+W3*W4 and leaf length. For these leaf dimensions contributions to their selection differentials due to correlations of these characteristics with other characteristics in the regression were often present. For

Table 2. Characterization of selection on two sets of characteristics of transplanted seedlings of P. major ssp. pleiosperma in different subsites at a beach plain. Selection differentials (S), the component of S due to direct selection (S_d), with the multiple r^2, and the corresponding levels of significance of the correlation coefficient and the partial correlation coefficient are given. Components of fitness: W_1, survival of seedlings until the first flowering; W_2, the number of seeds produced at this first reproductive stage; W_3, survival until the second stage of flowering; W_4, the number of seeds produced at the second and third stage of flowering. Levels of significance: ns not significant, * P<0.05, ** P<0.01, *** P<0.001; - no analysis performed.

Plant characteristic	Subsite 1				Subsite 2			
	W1		W1*W2+W3*W4		W1		W1*W2+W3*W4	
	S	$S_d(r^2=.28)$	S	$S_d(r^2=.21)$	S	$S_d(r^2=.16)$	S	$S_d(r^2=.43)$
Number of leaves (1985)	.2 *	.3 **	.2 ns	.5 *	.1 ns	.0 ns	.6 ***	.3 *
Leaf length (mm) (1985)	-.5 ns	-1.3 *	1.5 ns	.9 ns	.4 *	.4 ns	4.5 ***	3.4 ***
Leaf width (mm) (1985)	.0 ns	.0 ns	.6 *	.3 ns	.1 ns	-.0 ns	1.3 ***	.4 ns
Lamina/leaf length ratio (1985)	.01 ns	.02 ns	.02 ns	.00 ns	.00 ns	.00 ns	.00 ns	.00 ns
Grazing index (1985)	.1 ns	.2 *	.3 ns	.6 **	.0 ns	.0 ns	.0 ns	.0 ns
	W3		W3*W4		W3		W3*W4	
	S	$S_d(r^2=.75)$	S	$S_d(r^2=.29)$	S	$S_d(r^2=.53)$	S	$S_d(r^2=.35)$
Age of the plant (month) at first reproduction	-2.3 *	-2.2 ***	-1.6 *	-.1 **	-.4 *	-.1 ***	-.3 ns	-1.2 ***
Est. biomass of the leaves (mm^2) at first reproduction[1.]	.16 ns	.25 *	.49 ns	.53 ns	-.01 ns	.04 *	.39 *	.37 **
Biomass of the spikes (mg) at first reproduction[1.]	-.6 *	-.1 ns	-.2 ns	.1 ns	-.1 ns	.1 ns	.5 *	.5 **

Plant characteristic	Subsite 3			
	W1		W1*W2+W3*W4	
	S	$S_d(r^2=.25)$	S	$S_d(r^2=.35)$
Number of leaves (1985)	.2 ns	.1 ns	1.4 ***	1.2 *
Leaf length (mm) (1985)	4.5 ns	-11.3 *	-6.2 ns	-27.9 *
Leaf width (mm) (1985)	.7 ns	2.4 ns	4.5 *	4.3 ns
Lamina/leaf length ratio (1985)	.01 ns	-.00 ns	.05 ns	.02 ns
Grazing index (1985)	-.2 *	-.2 ns	-.2 ns	.0 ns
	W3		W3*W4	
	S	$S_d(r^2=1.00)$	S	$S_d(r^2=0.26)$
Age of the plant (month) at first reproduction	-2.4 ***	-	-2.4 ***	-2.5 ns
Est. biomass of the leaves (mm^2) at first reproduction[1.]	- .77 ns	-	- .87 ns	- .08 ns
Biomass of the spikes (mg) at first reproduction[1.]	.0 ns	-	-1.4 ns	-1.5 ns

1. Regression analysis after log-transformation.

example, at subsite 3 the direct component of the selection differential with respect to W1*W2+W3*W4 for the leaf length was -27.9 mm, whereas the total selection differential of this characteristic was only about -6.2 mm (corresponding correlation not significant). The components of this selection differential of leaf length due to correlations with number of leaves and with leaf width were 8.5 and 13.4 mm, respectively (not shown). Therefore, it is concluded that the cost of forming long leaves in terms of seed production is partly counter-balanced by positive effects of leaf width and leaf number on seed production.

At subsite 1 partial correlation coefficients between the grazing index and the components of fitness were significantly positive, suggesting that plants that were relatively heavily grazed had a higher fitness. However, this direct component of the selection differential was for the greater part neutralized due to correlations with morphological characteristics of the shoot. Only at subsite 3 loss of shoot material by grazing negatively influenced a fitness component (W1).

Selection on some life-history characteristics were studied on plants which survived until the second time of flowering; n=103 for subsite 1, n=218 for subsite 2, and n=51 for subsite 3. At all subsites selection differentials for the age of first flowering with respect to the fitness components survival until the second flowering (W3) and the subsequent seed production (W3*W4) were negative (Table 2, second part). Thus plants which reached the first reproductive stage in 1986, instead of 1985, had a lower probability of flowering again and also had a lower seed production a second and third time. For subsites 1 and 2 the partial correlation coefficients between the estimated shoot biomass at first reproduction and W3 were significantly positive. However, the corresponding simple regressions were not significant. Only at subsite 2 plants which had a relatively high leaf biomass at first reproduction produced more seeds in later reproductive periods. At subsite 1, the correlation between biomass of the spikes at first reproduction and W3 was significantly negative (the corresponding selection differential was -0.6 mg). Therefore, at subsite 1, in contrast to the other subsites, plants which produced a higher biomass of spikes at the first reproduction had a lower probability to reproduce a second time. This negative selection differential for the biomass of spikes at first reproduction was mainly due to the correlation with age at first flowering, resulting in a contribution to the selection differential of -0.5 mm^2. The correlation between spike production at first reproduction and W3*W4 was only significant at subsite 2. At this subsite plants, which had at first reproduction a relatively high biomass of spikes, produced more seeds in later reproductive periods than plants of which the first production of spikes was relatively low.

Table 3. Characteristics of lines of P. major ssp. pleiosperma at October 1985 (A) and at first reproduction (B) in a reciprocal transplant experiment at a beach plain. Lines originated from three subsites. F-values are given together with degrees of freedom (in parentheses) from a nested analysis of variance. Levels of significance: ns not significant, * P<0.05, ** P<0.01, *** P<0.001.

	Source of variation	Dependent variables			
		Number of leaves	Leaf length	Leaf width	Est. biomass of leaves[1.]
A.	Subsite	139.8 (2,715) ***	246.0 (2,54) ***	293.3 (2,804) ***	328.6 (2,715) ***
	Origin	1.4 (2,688) ns	0.8 (2,27) ns	3.2 (2,27) ns	2.6 (2,688) ns
	Line	1.0 (27,661) ns	2.4 (27,661) ***	1.8 (27,661) **	1.5 (27,661) ns
	Subsite x Origin	2.3 (4,715) ns	1.6 (4,54) ns	4.7 (4,804) ***	2.8 (4,715) *
	Subsit x Line	1.1 (54,661) ns	2.0 (54,661) ***	1.1 (54,661) ns	1.3 (54,661) ns
		Est. biomass of leaves[1.]	Number of spikes[1.]	Dry weight of spikes[1.]	Number of seeds[1.]
B.	Subsite	71.3 (2,54) ***	1.5 (2,402) ns	147.8 (2,453) ***	185.7 (2,380) ***
	Origin	1.7 (2,416) ns	2.3 (2,375) ns	0.5 (2,426) ns	0.5 (2,27) ns
	Line	1.5 (27,389) ns	0.5 (27,348) ns	0.7 (27,399) ns	1.6 (27,328) *
	Subsite x Origin	0.5 (4,54) ns	2.4 (4,402) *	1.5 (4,453) ns	1.8 (4,380) ns
	Subsite x Line	1.7 (54,389) ***	0.7 (54,348) ns	1.0 (54,399) ns	1.1 (52,328) ns

[1.] Analysis of variance after log-transformation.

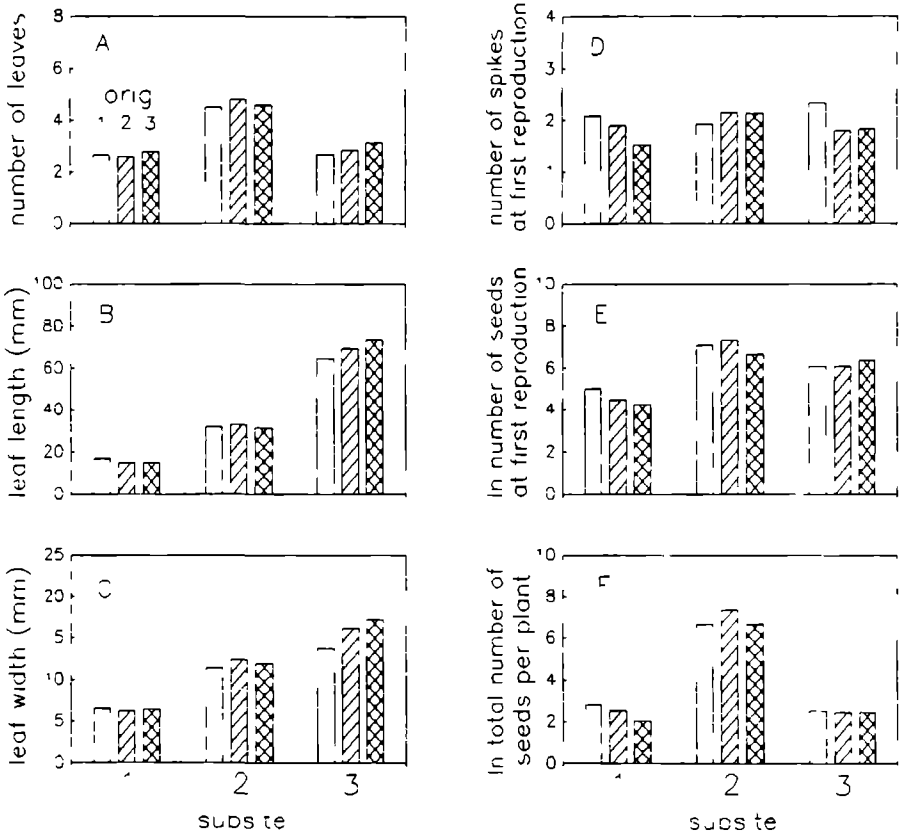
The median survival times of the transplanted seedlings of origin 1, 2, and 3 were: 17.3, 16.8, 17.8 months at subsite 1 and 5.2, 5.3, 5.6 months at subsite 3, respectively. At subsite 2 from each origin more than 50% of the plants were still alive at the end of the experiment. At this subsite the estimated time points at which 75% of the plants were still alive were for plants of origin 1, 2 and 3 11.6, 23.2 and 16.0 months, respectively. No significant differences between origins in survival curves could be detected, although there is a tendency that at subsite 2 plants of the "home" origin have a lower mortality rate than plants from origin 1 and 3. Differences between origins in survival rates of transplanted seedlings until the first and the second flowering were also non-significant.

Variation in vegetative and generative characteristics (with exception of the number of spikes at first reproduction) of transplanted plants can mainly be explained by subsite effects (Table 3, Fig. 1). At subsite 1 and 3 plants from all origins had fewer leaves than at subsite 2 (Fig 1A, each contrast $P<0.01$), whereas the numbers of leaves at subsite 1 and 3 were not significantly different. Plants at subsite 1 had significantly shorter leaves than plants at subsite 2 and 3 (Fig 1B, $P<0.05$ and $P<0.001$, respectively), while these leaves were also narrower (Fig 1C, $P<0.01$ and and $P<0.001$, respectively). In addition, plants at subsite 2 had shorter and narrower leaves than plants at subsite 3 ($P<0.01$ and $P<0.05$, respectively). Differences in lamina/leaf length ratio did not differ between subsites (not shown).

Besides this environmentally induced variation the results of this transplant experiment show for some plant characteristics genetic variation between origins, depending however on the subsite examined. This interaction origin x subsite is significant for the leaf width at the end of the first season and the number of spikes at first reproduction and almost significant for the number of leaves per plant ($P=0.06$). Analysis of the simple effects of origin within each subsite revealed only that at subsite 3 differences in leaf width between origins were significant. At this subsite, dominated by shrubs, plants of the "home" origin had broader leaves than plants from origin 1 (Fig 1 C, $P<0.001$). The interaction of origin x subsite of leaf width is attended with a similar subsite x origin interaction of the estimated biomass of leaves (Table 3).

The number of spikes per plant at first reproduction also shows a significant subsite x origin interaction. At subsite 1 plants from origin 3 had less spikes than plants from origin 2 and from origin 1 (Fig 1D, $P<0.05$ and $P<0.001$, respectively), whereas no differences were found at the other subsites. In contrast to this, the dry weight of spikes differed only between subsites (Table 3, means at subsite 1, 2 and 3: 11.6, 34.9 and

Fig 1 Vegetative and generative characteristics of P major ssp pleiosperma in a reciprocal transplant experiment at a beach plain Plants originated from three subsites Means were computed over ten lines for leaf chareacteristics only replicates that were alive in autumn 1985 were included, and for numbers of spikes and seeds at first reproduction only replica that flowered, the mean total number of seeds per plant was computed over the number of replicates per line at the start of the experiment (n=10)



45.0 mg, respectively). The seed production at first reproduction, too, was mainly influenced by the subsite (Table 3, Fig. 1E). No significant differences between plants of different origins could be detected for this fitness component.

At subsite 1 and 2 there seems to be a slight tendency that plants of the "home" origin had a higher total seed production (taken over three years, non-reproducing plants included) at subsite 1 and 2 (Fig. 1F). Because of

non-normality of this total seed production, this tendency was tested by Kruskal-Wallis one-way analysis of variance: P=0.14 and P=0.24, respectively. Significant differences in single seed weight between origins or subsites equally could not be detected.

Effects of the line or subsite x line represent genetic variation which is not related to the three subsites. These effects were present for length and width of leaves, the estimated biomass of the leaves and the number of the seeds at first reproduction.

DISCUSSION

Several authors have stressed the importance of evaluating adaptive values of life-history characteristics of plants in their natural habitat over the entire life cycle (Antonovics and Primack 1982, McGraw and Antonovics 1983, Watkinson and Gibson 1985). However, various problems may arise in determining the average number of descendants produced by a single seed. For example, mortality rates during seed dispersal and during the stay of seeds in the soil are difficult to estimate for most species. Furthermore, perennial species may produce seeds over longer periods of time than are covered by demographic studies. In the present paper selection was studied on 26 day old seedlings, that were reciprocally transplanted into three subsites. From the demographic study of naturally occurring plants it was concluded that during the period immediately upon emergence the mortality rate of seedlings was very high, compared to the mortality rates of the transplanted seedlings. This mortality of naturally occurring seedings was especially high in the shrubs of *Hippophae* (subsite 3). Moreover, after three years many transplants were still alive and might continue to produce seeds for several years. Therefore, it should be emphasized that results of the present study on *P. major* ssp. *pleiosperma* only refer to the specific stages of the life cycle which were investigated: the first three seasons after establishment. After three seasons no significant differences in survival between seedlings of different origins were found. Thus, there are no indications that lines from a specific origin will produce seeds over a longer period of time than lines from other origins.

Selection regimes

The present study demonstrates that in a relatively early part of a succession series of a primary beach plain differences in selection regimes exist between different subsites, e.g. in direction and strength of selection on specific plant characteristics, and in the life-cycle stage in which selection occurs. In the low-lying area (subsite 1) survival until first flowering is promoted by having more but shorter leaves, resulting in a more decumbent growth form. This specific growth form may be adaptive in the low, very open vegetation of subsite 1, because either the microclimate will be more favourable at near ground level or plants with a relatively erect growth form will probably be more susceptible to grazing by rabbits. However, selection on the grazing index indicates that plants that flowered were grazed more than those that died before flowering. From these results it is not clear, whether at this subsite grazing actually increases the probability of flowering. At the other subsites a similar direction of selection on growth form could not be demonstrated. On the small elevations (subsite 2) plants with relatively long leaves had a higher probability of flowering. Through overtopping by means of these longer leaves *P. major* ssp. *pleiosperma* probably competes better against grasses, that grow on these patches in a short dense sward.

Differences between subsites also existed throughout the whole part of the life cycle that was studied. At subsite 1 only directional selection for broader leaves could be demonstrated. At subsite 2, during this part of the life cycle, selection occurred for plants having a growth form of many, relatively large, leaves. At subsite 3 plants were selected that had relatively more leaves, which were relatively short but broad. In contrast to the grass sward of subsite 2, at subsite 3 plants, irrespective of their leaf length, are always affected by shade. In this shrub environment broad short leaves with relatively little need of support tissue may have higher photosynthetic capacity than long narrow leaves (Givnish 1987). Therefore, plants with these broad leaves may have higher reproductive outputs.

Age and size at first reproduction are thought to be primary life-history traits under selection in populations (e.g. Samson and Werk 1986, Stearns and Koella 1986). In the present study at all subsites plants which reached the first reproductive phase relatively late had a lower probability of flowering again and then had a lower seed production. At subsite 2, probably because of the relatively high level of interspecific competition in the dense grassy vegetation, plants which had a relatively high shoot biomass at first reproduction produced more seeds in later reproductive periods. At the other subsites a similar relationship between size at first reproduction and reproductive output in subsequent years could not be demonstrated. Hirose and Kachi (1986) concluded from a study on

Oenothera glazioiana that in an infertile environment this semelparous species, reproducing early and having a small size at flowering, could not maintain itself, because the resultant small fecundity could not compensate for a higher mortality coupled with early reproduction. From the present results of a perennial species it is concluded that fertility of the soil was not a factor which causes that early flowering or flowering at a relatively small plant size is coupled with a relatively low seed production in subsequent years. Nevertheless, in contrast to the other subsites, at the low-lying subsite 1 the plants demonstrated that a relatively high investment in reproductive tissue at first reproduction was correlated with a lower probabilty of survival until the next reproductive period. Therefore, these results show evidence of a cost of reproduction of *P. major* ssp. *pleiosperma* which is apparently only expressed in an environment in which plant growth is restricted by low fertility and water content of the soil. Reznick (1985) also summarized studies which demonstrated that costs of reproduction were only evident in environments where food availability was restricted or adults were subjected to extra stress.

Correspondence between patterns of selection and genetic variation

The following questions should be answered to relate the differences in regimes of contemporary selection with genetic variation between origins. Firstly, can we demonstrate genetic variation between lines from different origins? Secondly, in what characteristics, e.g. fitness components or morphological characteristics do we find such genetic variation? Finally, can we interpret the genetic differences as the result of selection, and if not, what facts may be the cause of patterns of contemporary selection and genetic variation that do not correspond?

In the reciprocal transplant experiment variation in characteristics of morphology and life-history were mainly explained by the subsite effects. Therefore, it is concluded that within the mosaic environment of the beach plain variation in these characteristics of *P. major* ssp. *pleiosperma* is primarly due to phenotypic plasticity. Although the results showed the tendency that at subsite 1 and 2 "home" origins had a higher total seed production, differences between origins in none of the fitness components were significant. Nevertheless, for leaf width, estimated biomass of leaves and number of spikes at first reproduction subsite x origin interactions were present. Only at subsite 3 did lines from origin 3 have relatively broader leaves than lines from the other origins. Thus, "home" lines appeared to have leaf forms which may, at least partly, be the result of the selection regime at this subsite, dominated by shrubs. However, though

differences in direction and strength of selection on leaf form were also demonstrated between subsite 1 and 2, these differences in selection regime were not coupled with genetic differentiation in leaf form between lines from these subsites. In addition, at subsite 1 lines from origin 1 and 2 had higher numbers of spikes at first reproduction than lines from origin 3. The present results do not demonstrate that at subsite 1 plants with higher number of spikes have a higher fitness. However, the higher number of spikes is possibly associated with a more prostrate inflorescence which might result in lower predation of spikes by rabbits. It is concluded that in this beach plain the demonstrated patterns of contemporary selection and genetic variation in *P. major* ssp. *pleiosperma* hardly correspond: only to a small extent for growth form and not at all for life-history characteristics.

In general, for several reasons direction and strength of contemporary selection regimes, determined in demographic studies, may not correspond with genetic differences. Firstly, genetic constraints within a population may prevent the evolution of optimal phenotypes (Falconer 1981, Silander 1985). For example, genetic variation within a population may be absent. The present results demonstrate, that, beside genetic variation between origins, leaf morphology and seed production differed between lines, representing genetic variation which is not related to distinct subsites. Therefore, it is concluded that, at least for these characteristics, in the beach-plain population absence of genetic variation will not have constrained local adaptation.

Secondly, differences between the groups of plants may be caused by random genetic drift. No conclusions can be drawn with respect to the occurrence of genetic drift and thus it cannot be excluded that genetic drift has influenced the population structure on this beach plain.

Thirdly, habitats may change in time and therefore past and contemporary selection regimes may differ in such a way that genotypes do not have higher fitness at their "home" subsites. Succession at the former beach plain probably has caused this change. Possibly, the process of local adaptation of *P. major* ssp. *pleiosperma* is slower compared to the rate of succession of the environment.

Fourthly, in the beach-plain population extensive gene flow may cause lines not to demonstrate local adaptation to a specific habitat. For example, they may pursue a performance to maximize the fitness at the subsite with the highest reproductive output or, alternatively, the highest average fitness over all subsites. From the demographic results it was concluded that in none of the subsites the maintenance of *P. major* ssp. *pleiosperma* depended critically on input of seeds from other subsites. Thus, the existence of gene flow between subsites cannot be indirectly demonstrated on account of population dynamics. Van Dijk (1985) estimated the mean

gene transport per generation within a population of *P. major* ssp. *major* to be only 0.11 - 0.35 m. It might be hypothesized that within the beach-plain population simular levels of gene flow occur. However, rabbit droppings contained viable seeds of *P. major* ssp. *pleiosperma* which germinated in a growth cabinet. Therefore, grazing of mature spikes by rabbits might increase dispersal of seeds. Quantifying the gene flow between subsites due to rabbit grazing is, however, rather difficult, because for that purpose detailed data on the behaviour pattern of these animals are required.

Fifthly, small-scale environmental variability may influence the relative fitness of an individual plant (cf. Hartgerink and Bazzaz 1984). Some individuals may have a higher fitness because of the quality of their microhabitat, which results in a relatively slow selection. Because in the present study variation in fitness components was mainly due to environmental variation, this last reason will be very important at the beach plain.

The results of the reciprocal transplant experiment demonstrated genetic variation, which has been related to a pattern of environmental variation within the mosaic environment. No genetic differences were found between lines from subsite 1 and 2. Both subsites form part of a small scale heterogeneous environment that varies over distances of only 0.5 - 3 m. Lotz and Spoormakers (chapter 8) demonstrated genetic differences between lines from the three subsites in levels of phenotypic plasticity in seed yield components. In a greenhouse experiment, these authors even found such differences between lines from subsite 1 and subsite 2.

From the present results it is concluded that in a mosaic environment at a primary beach plain with spatial variability in soil nutrients, water content of the soil, and vegetation structure a relatively low degree of population subdivision for *P. major* ssp. *pleiosperma* has been established within the last 20 years. In a subsequent paper (chapter 7) differences between the lines, used in the present study, in responses of growth and reproduction to variation in supply of nutrients and water content of the substrate will be studied under controlled conditions.

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CHAPTER 7

VARIATION IN LIFE-HISTORY TRAITS WITHIN A SUBDIVIDED POPULATION OF PLANTAGO MAJOR L. SSP. PLEIOSPERMA PILGER

3. RESPONSES TO NUTRIENT SUPPLY AND WATERLOGGING

L.A.P. Lotz and H. Olff

Summary. At a primary beach plain a mosaic environment was present with small-scale spatial variability in nutrient availability and water content of the soil, and in vegetation structure. In a greenhouse experiment responses in growth, morphology and flowering to various levels of nutrient supply and water content of the substrate, were studied on lines of *Plantago major* ssp. *pleiosperma* that originated from three subsites within the beach plain. Levels of phenotypic plasticity in these characteristics due to variation in nutrient supply were of the same order of magnitude as those in a reciprocal transplant experiment in the natural habitats. Plant dry weight and percentage of flowering were increased, whereas the leaf-area ratio was decreased by waterlogging.

Taken over all treatments, lines from a subsite dominated by shrubs had higher leaf-area ratios, but lower growth rates, as well as delayed flowering when compared to lines from more open subsites. No indications were obtained from which the conclusion migth be drawn that lines from any subsite were specially adapted to specific levels of nutrient supply or water content of the substrate. It is suggested that spatial variability in vegetation structure caused a population subdivision in life-history characteristics at the beach plain during primary succession.

From a correlation analysis between performances of lines in the greenhouse experiment and those in the transplant experiment, it was concluded that the experimental factors in the greenhouse reflected only to a small extent variation in selection regimes in the natural habitat. The absence of plant-plant interactions in the greenhouse might importantly contribute to this weak correspondence.

Key words: biomass allocation - morphology - age at first flowering -

INTRODUCTION

Structures of plant communities or populations can be analysed by studying responses of species or genotypes to specific environmental factors. For example, by relating the responses of plants to competitive ability or relative fitness, one may learn what factors influence the distribution of species or genotypes over e.g. environmental gradients or succession series (Austin and Austin 1980, Parrish and Bazzaz 1982, Tilman 1985). Compared to the amount of literature on variability in life-history characteristics between species or plants of different populations, up till now only few papers have been published on variability in these characteristics within a single population (Schlichting 1986). In respect of this within-population variability an important question is to what extent differences in life-history characteristics and in phenotypic plasticity in these properties can evolve as an adaptation to patterns of environmental factors fluctuating in time and/or space (Bradshaw 1965, Via and Lande 1985, Schlichting 1986).

Several authors (e.g. Antonovics and Primack 1982) stressed the importance of evaluating adaptive values of plant characteristics in their natural habitat using field transplants. However, because of the complexity of environmental heterogeneity of natural habitats, results of reciprocal transplant experiments offer in general little opportunity to understand to what specific environmental factors responses of plants are important in terms of fitness. Two approaches may be fruitful to maximize the ecological relevance of experimental data on plant responses. Firstly, one may vary a specific environmental factor (e.g. nutrient availability) in the natural habitat itself and study effects of these changes on plants that occur naturally (e.g. Chapin et al. 1986, Gibson 1988). Secondly, one may study responses of plants to environmental factors under controlled conditions and relate them to plant growth and fitness in the field. In general, levels of experimental factors are better maintained under controlled conditions and their effects are not influenced by factors which are correlated with experimental manipulations in the field. The latter approach was pursued in the present study for these reasons.

The present study is part of a research project on variability in life-history characteristics within a population of *Plantago major* L. ssp. *pleiosperma* Pilger that occurs on a former beach plain. *P. major* is a self-

compatible, wind pollinated perennial herb with a high self-fertilization rate (Van Dijk and Van Delden 1981). Van Dijk (1985) estimated the mean gene transport per generation within a population to be only 0.11 - 0.35 m. At the study site three subsites were distinguished that differed in availablity of nutrients and in water content of the soil (chapter 5) and in vegetation structure (Lotz and Olff 1988). Lotz et al. (chapter 6) demonstrated differences between these subsites in direction and strength of contemporary selection on specific characteristics of morphology and life history. From results of a reciprocal transplant experiment they concluded that in this beach-plain population variation in morphology and life-history was mainly due to phenotypic plasticity. However, genetic differences between lines from different subsites were also found, e.g. in biomass of leaves and in growth form.

In the present paper results are presented from a greenhouse experiment in which nutrient supply and water content of the substrate were varied. Vegetative and reproductive characteristics were investigated using lines, which were also studied in the reciprocal transplant experiment of Lotz et al. (chapter 6). Aims of the greenhouse experiment were: 1. assessing the influence of nutrient supply and waterlogging on growth, morphology and flowering of these lines; 2. relating differences in plant characteristics between lines of different origins to the pattern of environmental variation in the field; 3. correlating shoot characteristics of lines at specific treatment combinations to performances of the same lines in a reciprocal transplant experiment at the three subsites. The specific purpose of this last-mentioned correlation analysis was to investigate whether in this relatively young coastal environment fitness of transplanted seedlings of lines can be predicted from data on responses of those lines to two abiotic factors that are known to vary considerably in the natural habitat, i.e. nutrient supply and level of water content of the substrate.

MATERIALS AND METHODS

Population description

The investigated population of *P. major* L. ssp. *pleiosperma* Pilger is located at a beach plain on the south-west coast of the Netherlands. This beach plain was embanked in 1966. At a study site of about 2,000 m^2 variation between three subsites in nutrient availability and water content of the soil has been described in detail by Troelstra et al. (chapter 5). In

addition, the vegetation of these subsites differs in structure (Lotz and Olff 1988, chapter 6). In summary, characteristics of the three subsites are: Subsite 1 is a low-lying area; the soil is low in nutrients and frequently water saturated. The nitrogen mineralization potential, determined in the laboratory on intact soil cores, was relatively low (ca. 35 mg $N.L^{-1}$ of soil, after nine weeks of incubation). This area is flooded after heavy rain and during winter; the vegetation is low (<0.05 m) and very open. Subsite 2 consists of small elevations (0.5-1.5 m in diameter) spread over at subsite 1; these patches are covered with a short, dense, grassy vegetation; the soil is relatively high in nutrients. The nitrogen mineralization potential was also relatively high (ca. 130 mg $N.L^{-1}$ of soil, after nine weeks incubation). Subsite 3 consists of relatively large patches (20-40 m in diameter), covered by shrubs of *Hippophae rhamnoides* L. At this subsite nutrient availability is also higher (e.g. the nitrogen mineralization potential was ca. 95 mg $N.L^{-1}$ of soil, after nine weeks of incubation) and water content is lower than in the soil of subsite 1; plants of *P. major* ssp. *pleiosperma* are shaded by the shrubs.

Greenhouse experiment

On April 25 1985, seeds of six lines (chapter 6) from each subsite were sown in containers with moist dune sand in a greenhouse (c. 20°C). At least 95% of the seeds of each line germinated within three days. Twenty days after sowing, seedlings were randomly selected from the stock of each line and transplanted into pots with quartz sand (height of pot 9 cm, contents 0.42 l, one plant per pot). At this day the differences in shoot dry weight between seedlings of lines from different origins (subsites) were not significant (nested analysis of variance, $F=1.7$; $d.f=2,17$; $P=0.21$). For three days after the transplanting the seedlings were sprayed regularly with water to minimize transplant effects. Three nutrient levels and two water levels were applied, together forming a full factorial treatment combination. Nutrient levels were created by adding different quantities of a solution twice the strength of the solution described by Steiner (1968): plants in the Low treatment received 2.6 ml solution per pot, the Intermediate treatment 38 ml, and the High treatment 75 ml. Previous experiments (Lotz and Blom 1986) generated the expectation that the biomass accumulation at these nutrient levels would be within the same range as that found at the study site. Micronutrients were supplied to each plant by adding 75 ml solution containing micronutrients with concentrations twice as high as described by Smakman and Hofstra (1982). The high water content (treatment "Waterlogging") was created by placing the pots in low containers with water (the water level was kept

permanently at 4 cm from the top of the pots). The sand substrate was saturated with water that entered the pots through holes in the bottom. In the pots with low water content (treatment "Draining") water was added as required to maintain soil moisture at c. 20% by weight.

On day 36 and 46 after sowing the number of flowering plants was determined for each treatment combination. On day 46 after sowing the following characteristics were measured for each individual plant: the number of leaves and spikes, the length and the width of the largest leaf (further referred to as leaf length and leaf width, respectively), and the length of the lamina of this largest leaf. Six replicates were used for this non-destructive assesment of plant characteristics. On the same day, one randomly selected plant was harvested from each line for each treatment combination. The following characteristics were measured for these harvested plants: fresh and dry weights of leaves, spikes and roots, and the leaf area. Leaf-weight ratio (LWR) was computed as the dry weight of leaves per total plant dry weight, the specific leaf area (SLA) as leaf area per dry weight leaves, and leaf-area ratio (LAR) as leaf area per total plant dry weight.

Reciprocal transplant experiment

At the start of the greenhouse experiment the remaining seedlings, which had not been transplanted into pots, were exposed to outdoor conditions. After four days 30 seedlings were randomly selected from each line and transplanted into a randomized complete block design; ten square plots (blocks) spread over each subsite with a single replicate of each line per plot. This reciprocal transplant experiment is described in detail by Lotz et al. (chapter 6). For the purpose of correlating performances of lines in the greenhouse and in the field, results of six lines per origin (the same lines as used in the greenhouse experiment) will be presented. On 2 October 1985 (159 days after sowing) the following characteristics of surviving plants were determined in the same way as the non-destructive determination in the greenhouse: the number of leaves (N), the length (L) and the width (W) of the largest leaf, the length of the lamina of the largest leaf, and whether a plant flowered or not. Biomass of the leaves was estimated by multiplying N by L by W. This product is highly correlated with the dry weight of the leaves of *P. major* (Lotz and Blom 1986). In October 1985 and in September and October 1986 and 1987 mature spikes were harvested and the number of seeds per plant was determined.

Data analysis

For the greenhouse experiment differences between plants of different origins in survival and flowering were tested by fitting a loglinear model (BMDP-programme PLR, Dixon 1985). Data of growth, morphology and reproductive output (non-flowering plants excluded for reproductive traits) were analysed with analysis of variance (SPSS-subprogramme MANOVA, Nie and Hull 1981) with as independent variables: supply of nutrients, level of water content, origin, and -in case of the non-destructively determined characteristics- line nested within origin. Non-significant mean squares were pooled with the within-cells mean squares when appropriate. Comparisons of means between origins were made by *a priori* simple contrasts. Comparisons of means over treatments were made by performing Tukey's honestly significant difference method.

Correlations between performances of lines in the greenhouse and the field were tested by computing Kendall's coefficient of rank correlation between mean values per line per treatment combination in the greenhouse and in the reciprocal transplant experiment (n=18). For this correlation analysis three greenhouse treatment combinations were selected: "Low nutrients and draining", "Low nutrients and waterlogging" and "High nutrients and draining". Based on the description of the environment of the study site by Troelsta et al. (submitted) we *a priori* concluded that the first two treatment combinations correspond best with the environment of subsite 1 and the third treatment combination with the environments of subsite 2 and 3.

RESULTS

Greenhouse experiment

First we will consider the main and two-way interaction effects of the different treatments. Nutrient supply influenced all plant characteristics determined on day 46 after sowing (Table 1, Fig 1). At the low nutrient treatment, plants had lower dry weights than plants at the intermediate and high nutrient treatments ($P<0.001$). The lower plant dry weight at the low nutrient treatment was coupled with a lower LWR ($P<0.01$) and a lower LAR ($P<0.01$). The lower the supply of nutrients, the lower the number of leaves, the size of the leaves and the number and dry weight of spikes was also reduced. Although main effects of nutrient treatment were significant

Fig. 1. The effect of nutrient supply and water saturation of the substrate on growth, morphology and flowering of P. major ssp. pleiosperma in a greenhouse. Plants originated from three subsites within a beach plain. Means were computed over six lines with one harvested plant each (A) or with 6 replicates for non-destructively determined characteristics. For dry weight and number of spikes only flowering plants were included.

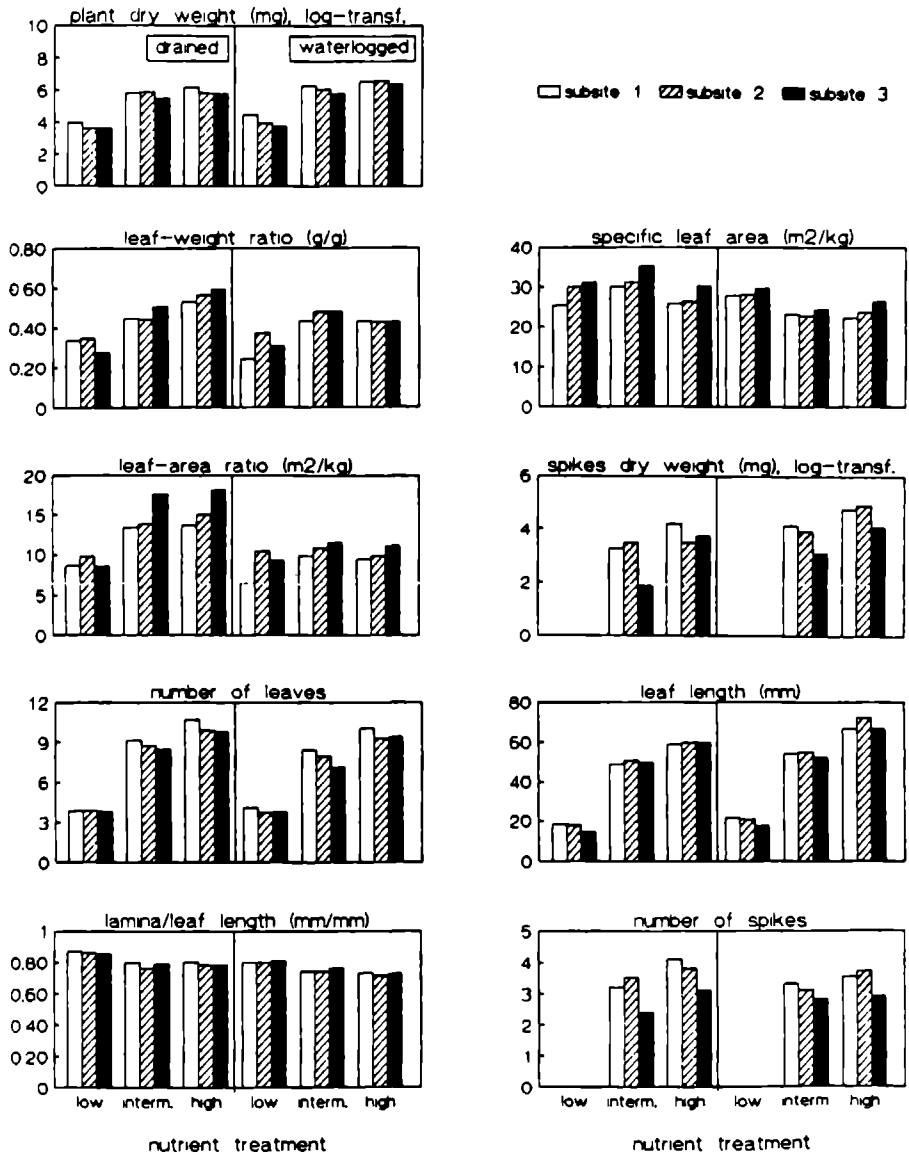

Table 1. Effects of nutrient supply and level of water saturation on vegetative and reproductive characteristics of P. major ssp. pleiosperma in a greenhouse. Lines originated from three origins (subsite 1, 2, and 3) within a beach-plain population. Effects were tested in a (in case of B: nested) analysis of variance. F-values are given, together with degrees of freedom (in parentheses). In the analysis of generative characteristics data from the low nutrient supply were excluded because of empty cells. Abreviations: Nut nutrient supply, Wat level of water saturation, Line inbred line, Orig origin. Levels of significance: ns not significant, * P<0.05, ** P<0.01, *** P<0.001.

	Source of variation	Dependent variables	
A.		Plant dry weight[1]	Leaf-weight ratio
	Nut	170.2 (2,89) ***	42.7 (2,89) ***
	Wat	15.0 (1,89) ***	8.9 (1,89) **
	Nut x Wat	0.7 (2,89) ns	6.4 (2,89) **
	Orig	5.2 (2,89) **	1.6 (2,89) ns
	Nut x Orig	0.4 (4,89) ns	1.1 (4,89) ns
	Wat x Orig	0.1 (2,89) ns	0.6 (2,89) ns
	Nut x Wat x Orig	0.4 (4,89) ns	1.1 (4,89) ns
B.		Number of leaves	Leaf length
	Nut	1307.4 (2,562) ***	2796.9 (2,30) ***
	Wat	26.1 (1,562) ***	1106.3 (1,30) ***
	Nut x Wat	8.0 (2,562) ***	12.8 (2,30) ***
	Orig	4.3 (2,15) *	2.0 (2,15) ns
	Nut x Orig	2.8 (4,562) *	1.2 (4,30) ns
	Wat x Orig	0.5 (2,547) ns	1.3 (2,15) ns
	Nut x Wat x Orig	0.8 (4,562) ns	1.2 (4,30) ns
	Line	3.6 (15,532) ***	8.7 (15,532) ***
	Nut x Line	1.2 (30,532) ns	3.3 (30,532) ***
	Wat x Line	0.8 (15,532) ns	2.6 (15,532) **
	Nut x Wat x Line	1.4 (30,532) ns	1.8 (30,532) **

1. Analysis of variance was performed after log-transformation.

Specific leaf area	Leaf-area ratio	Spikes dry weight[1]
6.2 (2,88) **	17.0 (2,88) ***	14.6 (1,59) ***
37.7 (1,88) ***	26.7 (1,88) ***	11.7 (1,59) ***
13.6 (2,88) ***	5.8 (2,88) **	0.1 (1,59) ns
10.4 (2,88) ***	5.0 (2,88) **	5.6 (2,59) **
0.4 (4,88) ns	1.0 (4,88) ns	1.3 (2,59) ns
1.6 (2,88) ns	0.5 (2,88) ns	0.1 (2,59) ns
0.6 (4,88) ns	0.7 (4,88) ns	1.4 (2,59) ns
Leaf width	**Lamina/leaf length**	**Number of spikes**
1945.5 (2,30) ***	85.6 (2,562) ***	46.1 (1,356) ***
8.3 (1,30) **	113.4 (1,562) **	1.7 (1,356) ns
6.0 (2,30) **	2.3 (4,562) ns	2.4 (1,356) ns
2.1 (2,15) ns	0.8 (2,15) ns	5.3 (2,15) *
0.8 (4,30) ns	0.6 (4,30) ns	0.3 (2,15) ns
1.0 (2,547) ns	1.0 (2,15) ns	2.3 (2,15) ns
1.2 (4,30) ns	0.6 (4,562) ns	4.8 (2,356) ***
4.8 (15,532) ***	1.2 (15,532) *	9.9 (15,341) ***
2.4 (30,532) ***	1.2 (30,532) ns	1.9 (15,341) *
1.6 (15,532) ns	1.7 (15,532) *	1.7 (15,341) *
1.5 (30,532) *	1.1 (30,532) ns	1.5 (15,341) ns

for the SLA and the ratio lamina/ leaf length, *a posteriori* contrasts between nutrient treatments lumped over water treatments were not significant for these characteristics. In addition, on day 36 and 46 after sowing the number of flowering plants was highest in the higher nutrient treatments (Table 2, $P<0.001$). On day 36 after sowing, no plants were flowering at the low nutrient level.

The water content of the substrate had also a great effect on the characteristics studied, with the exception of the number of spikes per plant (Tables 1 and 2, Fig. 1). Waterlogged plants had, on average, slightly higher dry weights than plants in the drained treatment, but had a lower LWR. This lower biomass allocation to leaves of the waterlogged plants was coupled with both a lower SLA and LAR. In addition, waterlogged plants had slightly fewer leaves, which were relatively longer, but narrower -especially at the intermedate and high nutrient supplies- (means of the leaf width not shown). These longer leaves had shorter laminae compared

Table 2. Flowering of P. major ssp pleiosperma, originating from three subsites within a beach plain, in a greenhouse at day 36 (A) and 46 (B) after sowing. Mean percentages of flowering were computed over six inbred lines with each six replicates. Greenhouse treatments: L = Low nutrients, I = Intermediate nutrients, H = High nutrients, D = Draining, W = Waterlogging.

	Origin	Greenhouse treatments					
		D			W		
		L	I	H	L	I	H
A	Subsite 1	0	17	42	0	61	72
	2	0	17	39	0	50	81
	3	0	0	14	0	33	42
B	Subsite 1	6	92	100	13	100	100
	2	9	100	97	33	97	97
	3	9	89	83	8	97	97

to the total length. In the intermediate and the high nutrient treatments drained plants flowered less frequently than waterlogged plants (Table 3A, $P<0.001$). Compared to the drained treatment, waterlogging also caused higher dry weights of spikes (Table 1A, Fig. 1).

For several plant characteristics the interaction between nutrient supply and water treatment was significant (Table 1). At the treatment draining, the LWR increased with the nutrient supply ($P<0.05$), while at the treatment waterlogging differences in LWR between high nutrient plants and low nutrient plants were not significant. The reduction of SLA due to waterlogging was stronger for plants at the intermediate nutrient level ($P<0.01$) than for other plants. In addition, the increase in LAR due to a higher nutrient supply was smaller for waterlogged than for drained plants ($P<0.01$). Though for the number of leaves per plant the interaction nutrient supply x water treatment was significant, *a posteriori* contrasts did not demonstrate significant differences between cell means. The increase in leaf length due to a higher nutrient supply was higher in waterlogged than in drained plants. For the percentage of flowering plants no significant interaction between nutrient supply and water content existed. To summarize, higher nutrient levels promoted both plant dry weight (coupled with a higher LAR) and reproduction, and most size-related traits varied accordingly. Waterlogged plants had longer, but narrower leaves, and a slightly higher dry weight and reproductive output. The drained plants, however, had a higher LWR, SLA and LAR, but these differences disappeared at low nutrient levels.

We will now consider differences between lines in their reactions to the different treatments. Lines from the low area (subsite 1) had over all treatments a higher dry weight than lines from the shrubs (subsite 3) (Fig 1, Table 3). In addition, plants from subsite 2 also had a higher dry weight of the leaves than plants from subsite 3 (Table 3). The lower plant dry weight of lines from subsite 3 was coupled with a higher SLA and, only when compared to lines from subsite 1, with a higher LAR (Fig. 1, Table 3). Differences in percentage dry matter were not significant between plants from different origins (data not presented). Plants from subsite 1 had more leaves than plants from subsite 3. In addition, both on day 36 and 46 after sowing, less plants from subsite 3 were flowering compared to plants from subsites 1 and 2 (in each case $P<0.001$). On day 46, after sowing plants, from subsite 3 had less spikes and lower spike dry weights than plants from subsite 1 and 2 (Table 3).

The results of the greenhouse experiment demonstrate that only for the number of leaves and the number of spikes per plant a significant difference was found between the three origins in their response to the treatments (Table 1). The higher the nutrient level was, the more the

Table 3. A priori contrasts between plants from three different origins (subsites) in a greenhouse experiment. Plant characteristics were selected for which effects due to origin were significant in the analysis of variance (Table 1). ns not significant, * P<0.05, ** P<0.01, *** P<0.001.

Dependent variable	Origin means			Contrasts between origins		
	1	2	3	1 - 2	1 - 3	2 - 3
Plant dry weight (mg)[1]	5.58	5.36	5.12	ns	**	ns
Leaf dry weight (mg)[1]	4.61	4.50	4.27	ns	***	*
Specific leaf area (m^2/kg)	25.6	26.8	29.3	ns	***	**
Leaf-area ratio (m^2/kg)	10.3	11.7	12.8	ns	**	ns
Spike dry weight (mg)[1]	3.41	3.51	3.17	ns	**	*
Number of leaves	7.7	7.2	7.1	ns	*	ns
Number of spikes	3.4	3.4	2.8	ns	*	*

1. Analysis of variance was performed after log-transformation.

number of leaves of plants from subsite 1 exceeded that of plants from subsite 3. Moreover, the differences between origins in the number of spikes per plant depended both on the nutrient and the waterlogging treatment. Although, taken over the intermediate and the high nutrient treatments, plants from subsite 1 had a higher number of spikes than plants from subsite 3, this difference in spike number was smaller in the treatment combination "intermediate nutrients and waterlogged" than in the other treatment combinations.

Tabel 1 B shows for the number of leaves, leaf length, leaf width, the ratio lamina/ leaf length, and the number of spikes significant effects of line within origin, in part dependent on treatments. These effects represent genetic variation which is not related to the distinct subsites.

To summarize, plants from subsite 1 had a higher dry weight than plants from subsite 3, and size-related traits varied accordingly. However, plants from subsite 3 had a higher SLA and LAR. The size of plants from subsite 2 was intermediate between that of plants from subsite 1 and subsite 3. Plants from subsite 1 and 2 flowered earlier than plants from subsite 3. Finally, for various plant characteristcs genetic variation that is not related to the distinguished subsites, is demonstrated.

Correlations between performances of lines in the greenhouse and in the field

Rank correlations between shoot characteristics of lines in the greenhouse and the performance of these characteristics and a component of fitness in the reciprocal transplant experiment are listed in Table 4. The following patterns of significance of correlations can be distinguished. Only a single correlation existed between the plant characteristics determined in the greenhouse and those in the field. No significant correlations at all were found between plant performances in the greenhouse and in the small patches of subsite 2. However, the length and width of the leaves, the estimated biomass of the leaves, and the ratio lamina/ leaf length in the greenhouse treatment combination "low nutrients and drained" were correlated with the total seed production per plant in the low area (subsite 1). Thus, lines, that in this greenhouse treatment combination had larger leaves and relatively shorter laminae, produced relatively more seeds at subsite 1. In addition, the number of leaves, leaf length and width and the estimated biomass of the leaves in treatment combination "low nutrients and draining" and the number of leaves in the treatment combination "low nutrients and waterlogging" were positively correlated with the total seed production per plant at subsite 3. The ratio lamina/ leaf length in the treatment combination "high nutrient and draining" was also positively

Table 4. Kendall's coefficients of rank correlation between performances of inbred lines in a greenhouse and in a reciprocal transplant experiment (n=18). Characteristics in the field: X = the corresponding plant characteristic at the end of the first season, W_{seeds} = total seed production per plant in 1985-1987 (non-flowering plants included). Greenhouse treatments: L = Low nutrients, H = High nutrient, D = draining, W = waterlogging. Significant rank correlations are indicated: * P<0.05, ** P<0.01, *** P< 0.001.

Greenhouse		Subsite 1		Subsite 2		Subsite 3	
Plant characteristic	treatment	X	W_{seed}	X	W_{seed}	X	W_{seed}
Number of	LD	- .06	.27	.03	- .03	- .17	.35 *
leaves	LW	- .03	.14	- .10	- .09	- .06	.34 *
	HD	- .16	.25	- .02	.14	- .14	.26
Leaf length	LD	.13	.38 *	- .16	.20	- .08	.45 **
	LW	.20	.15	.00	.20	.03	.12
	HD	- .05	.21	- .17	.07	.02	.19

Leaf width	LD	- .08	.44 **	.09	.26	- .09	.52 **
	LW	- .27	.00	- .02	- .07	- .23	.08
	HD	- .22	.01	.05	- .12	- .17	.16
Lamina/leaf	LD	.35 *	- .34 *	.15	- .27	- .28	- .20
length	LW	.07	.06	.10	.06	.17	.10
	HD	.08	.11	.21	.05	.18	.32 *
Est. biomass	LD	.19	.37 *	- .07	.12	- .10	.50 **
of leaves	LW	.27	.18	- .19	.04	- .07	.27
	HD	.22	.21	- .09	.07	.05	.25

correlated with the seed production per plant at subsite 3. Thus, lines that had a relatively long lamina compared to the leaf length in the greenhouse, produced more seeds in this environment dominated by shrubs.

DISCUSSION

Responses to environmental factors

As was to be expected, the higher the supply of nutrients, the higher the dry weight and the greater the dimensions of both leaves and spikes of plants of *P. major* ssp. *pleiosperma*. The increase in biomass with a higher nutrient supply was associated with an increase in the LAR. The latter increase was coupled with a higher LWR, i.e. a relatively higher investment of biomass in photosynthetic tissue. Variation in the LAR due to nutrient supply could not be explained by differences in the SLA (cf. Hirose 1988).

The plastic responses to variation in nutrient supply, that have been established in the present greenhouse experiment for shoot dimensions and flowering, are of the same order of magnitude as those observed with the same lines in the recipocal transplant experiment over the three subsites (chapter 6). For example, at the end of the first season the mean length of the largest leaf, taken over all lines, ranged from about 17 mm in the low area (subsite 1), to about 75 mm in the shrubs (subsite 2), whereas the leaf length ranged in the present experiment from about 18 mm (treatment low nutrient supply) to 65 mm in the high nutrient treatment. In addition, at subsites 2 and 3 plants from all origins started to flower about 3 weeks earlier than plants at subsite 1 (data not presented), whereas in the greenhouse at the intermediate and the high nutrient level plants started to flower about ten days earlier than at the low nutrient level.

It seems likely that the lower biomass of shoots of *P. major* ssp. *pleiosperma* at subsite 1, as compared to the other subsites, is not due to negative effects of a high water content of the substrate. In the waterlogged treatment plants had even higher dry weights than in the drained treatment. This result agrees with data of Schat (1984) for *Plantago coronopus* and *Samolus valerandi*. These species are also abundantly present at subsite 1. This author demonstrated that both these species, compared to several other species from dune slacks, could maintain a higher photosynthetic activity under waterlogged conditions and showed a high degree of root porosity. Moreover, in a substrate with a

high water content nutrient availability might be higher because of a higher transport, e.g. of P (Nye and Tinker 1977, Wild 1981), which may be relevant to plants of which the growth is nutrient-limited. Thus, at subsite 1 the low growth rate of plants is probably caused by a low nutrient availability. In addition, soils with high water contents have lower soil temperatures, which fact may decrease root activity and mineralization rates and thus, plant growth.

The higher plant dry weight in the waterlogged treatment was associated with a lower LAR and a higher percentage of flowering than in the drained treatment. Both components of the LAR, the LWR and the SLA, were lower at the high water content. The higher the nutrient supply, the stronger was the reduction of the LWR due to waterlogging. For the SLA a similar interaction of the nutrient and water treatments was observed. The effect of waterlogging on the pattern of biomass allocation to shoot and root differs between species. For many species shoot-root ratios have been reported to be higher or equal at high than at intermediate water content of the soil (Jones and Etherinton 1971, Trought and Drew 1980, Schwintzer and Lancelle 1983). However, Jones and Etherington (1971) reported a decrease of shoot-root ratio for *Carex nigra*, and Talbot et al. (1987) a reduction of both the SLA and the leaf area per plant for *Salix caprea* by waterlogging.

Waterlogging affected also some characteristics of the growth form. Compared to the drained plants, plants in the waterlogged treatment had fewer leaves which were more erect and were longer and relatively narrow and had a short lamina relative to the leaf length. This change in orientation and form of leaves has been described for *P. major* by Ridge (1987) and is probably controlled by ethylene. This gas accumulates in waterlogged plants and promotes cell elongation, especially in petioles (Ridge 1987, Voesenek and Blom 1989). In the case of flooding, elongation of petioles may result in the rise of submerged leaves above the water surface. In contrast to the greenhouse results, in the reciprocal transplant experiment plants at subsite 1 did not have a lower ratio lamina/ leaf length than plants from subites 2 and 3 (chapter 6). The difference in pattern of biomass allocation to petioles and laminae in waterlogged soil, between plants in the greenhouse experiment and plants in the field, might be explained by the fact that in a dune slack vegetation, neighbouring plants (e.g. Juncus-species) may to some extent alleviate effects of waterlogging (Schat 1984). Moreover, the increase in the leaf length was particularly strong in the high nutrient treatment and much less important in the low nutrient treatment. Therefore, it is unlikely that water saturation of the substrate will considerably affect leaf length in the nutrient poor soil of subsite 1.

In the greenhouse experiment the presence of genetic variation for several plant characteristics has been demonstrated. This genetic variation has, partly, been related to an *a priori* distinguished pattern of environmental variation at the beach plain. On day 46 after sowing, lines from the low area (subsite 1) had higher dry weights of both total plant and shoot than lines from subsite 3. It was remarkable that the lower biomass of lines from subsite 3, as compared to the lines from subsite 1, was associated with a higher LAR, whereas differences in percentage dry matter between origins were not significant. So, in the greenhouse the relatively smaller plants from the subsite dominated by shrubs, had a higher investment in photosynthetical active area than plants from the unshaded habitat of subsite 1. This higher LAR was explained by a higher SLA of plants from subsite 3. Comparing species (Björkman 1981, Givnish 1987) or ecotypes (Björkman and Holmgren 1963) plants from a shady environment demonstrated higher SLA than plants of exposed environments. When other resources are not limited, plants with relativey higher SLA should have higher RGR, and thus a higher competitive ability, in environments with low light intensities (Björkman 1981). Indeed, in the reciprocal transplant experiment lines from origin 3 had a higher biomass of leaves, and broader leaves than lines from origin 1 and 2 in the shady environment of the *Hippophae*-shrubs (chapter 6). These authors suggested that the differences in leaf form, too, may be the result of differences in selection regimes due to variation in levels of shading.

In addition, lines from subsite 1 and 2 flowered earlier than lines from origin 3 in the greenhouse. In the reciprocal transplant experiment plants from origin 1 and 2 did not flower in an earlier year than plants from origin 3. However, differences in time of onset of flowering within the growing season have not been studied in the field.

The non-parametric correlations with performances of lines in the greenhouse (Table 4) demonstrated that both in subsites 1 and 3 total seed production of transplanted plants was positively correlated with the leaf dimensions in the treatment combination "low nutrients and drained". The length, width and biomass of the leaves in the high nutrient treatment were not significantly correlated with this fitness component. This fact suggests that both at subsite 1 and 3 fitness of plants may be predicted from a potential rate of biomass allocation to the shoot at low nutrient availability. This result partly corresponds to the selection differentals, which were determined in the transplant experiment for leaf dimensions with respect to total seed production. However, in the latter study number of leaves and leaf length and width were also positively correlated with seed production at subsite 2, whereas these correlations were not found

between performances of lines in the greenhouse and the transplant experiment. Moreover, only one correlation was found between characteristics, determined in the greenhouse, and the corresponding characteristics in the field. Therefore, it is concluded that the experimental factors investigated in the greenhouse experiment reflect only to a small extent the environmental conditions, that plants of *P. major* ssp. *pleiosperma* have to cope with in the natural habitat. The absence of plant-plant interactions in the greenhouse might importantly contribute to this weak correspondence.

The results of the present study on lines of *P. major* ssp. *pleiosperma* demonstrated levels of phenotypic plasticity in growth, morphology and flowering, which are of the same order of magnitude as have been determined in the natural habitat. No indications were obtained from which the conclusion can be drawn that in the mosaic environment of the beach plain lines from any subsite were specially adapted to specific levels of the experimental factors nutrient supply and water content of the substrate. However, from both the results of the reciprocal transplant experiment (chapter 6) and the present study it is concluded that some population subdivision has been established within a period of 20 years of primary succession. In both studies genetic differences in vegetative and reproductive characteristics could be demonstrated to exist between the low area and the small grassy patches on the one hand and lines from the shrubs on the other hand. Both selection differentials for leaf morphology (chapter 6) and mean values of the characteristics, for which this population structure was shown to exist (i.e. leaf morphology, growth, and precoccity), provide evidence that selection in the shady environment of the shrubs has led to genetic differentiation. Therefore, it is suggested that during primary succession at the primary beach plain with spatial environmental variability in both nutrient availability and water content of the soil, and in vegetation structure, the latter biotic factor has constituted a selective pressure, that has caused a population subdivision for morphological and life-history characteristics. Of course, the development of the specific pattern of vegetation structure might, in turn, depend strongly on the spatial variation in abiotic factors.

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CHAPTER 8

DIFFERENTIATION IN REPRODUCTIVE CHARACTERISTICS WITHIN A POPULATION OF PLANTAGO MAJOR L. SSP. PLEIOSPERMA PILGER

L.A.P. Lotz and L.D.H. Spoormakers

Summary. Reproductive characteristics of inbred lines originating from three different subsites within a population of *Plantago major* L. ssp. *pleiosperma* Pilger were studied in a greenhouse experiment at two nutrient levels. Inbred lines originating from subsites dominated by *Hippophae rhamnoides* L. had lower seed production than plants originating from the surrounding grassland, whereas differences in reproductive effort were not significant. The mechanism of seed-yield regulation was studied on seven yield components. Plants originating from the site dominated by the shrubs showed higher levels of plasticity in the number of flowers per fructiferous spike. In addition, differences in plasticity in the number of capsules per flower were observed in plants originating from a fine-scale mosaic environment with spatial variation over distances of 1 m. It was concluded that plants from microhabitats where the fluctuation of environmental factors was relatively unpredictable, demonstrate a stronger tendency for regulating investment in reproduction in later stages of the sequence of yield components than plants from a more predictable microhabitat.

Key-words: Micro-differentiation - Mosaic environment - Reproduction - Seed-yield components - Plasticity - *Plantago*.

INTRODUCTION

The relationship between genetic polymorphism and heterogeneous environments is of current interest in population genetics and population biology (e.g. Hedrick 1986). Most data on within-population variation are based on electrophoretic studies. Experimental studies on causes of microgeographical differentiation of morphological, physiological or life-history characteristics in plant populations are rather scarce. In order to understand the adaptation of plants to their environment, data on genetic variability for phenotypic plasticity in such characteristics within a population are also needed (Schlichting 1986).

The genetic structure of plant populations may be influenced by e.g. selective forces, rates of gene flow, and random factors. When coupled with restricted gene flow, spatial variation in selective forces may stimulate local differentiation (Fowler and Antonovics 1981, Ray and Jain 1982, McGraw and Antonovics 1983). If, on the contrary, the scale of spatial environmental heterogeneity is small compared to the mean distance of gene flow (i.e. the progeny of an individual plant experiences two or more possible habitats) there may be a selection for high levels of phenotypic plasticity (Bradshaw 1965, Caswell 1983, Via and Lande 1985). In this respect many questions are still open (Werner 1985). For instance, what (combinations of) environmental factors select for degrees of plasticity, on what spatial scale and in which part of the life history of a plant does microdifferentiation occur, and what factors act as constraints to the process of evolutionary divergence?

A research project was started in 1984 to investigate the relationship between small-scale environmental heterogeneity and the variation in life-history characteristics within a population of *Plantago major* L. ssp. *pleiosperma* Pilger. Spatial variation in environmental factors was studied in detail at a former beach plain (chapter 5). In this study site a mosaic environment is present in which there is spatial variation in the nutrient availability and water saturation of the soil, and vegetation structure. At three subsites in this mosaic environment demographic studies on naturally occurring *P. major* plants demonstrated marked differences in life-history characteristics, e.g. in survival rates, biomass, flowering time and reproductive output (chapter 6, 7).

In the subsite with a low nutrient availability survival rates were relatively high, but seed yield per plant was low. In two subsites with higher nutrient availabilities survival rates were relatively low, probably due to periodic summer droughts and interspecific competition. In the latter sites plants which survived until the end of August had relatively high seed yields. In these subsites with high nutrient availability the degree

of unpredictability of fluctuations in growth conditions is supposed to be higher than in the nutrient-poor subsite.

P. major is a self-compatible, wind pollinated species with a high rate of self-fertilization (Van Dijk 1985). Seed yield (the total weight of seeds per plant) in this species is strongly influenced by nutrient availability (Lotz and Blom 1986). In the present study seed yield in *P. major* was divided into the following yield components: initiated spikes per plant, fructiferous spikes per initiated spike, flowers per fructiferous spike, capsules per flower, ovules per capsule, seeds per ovule, and single seed weight. By multiplying these seven components the total weight of seeds produced by a plant is obtained. Each component represents a possible unit of plastic response. Altering any component may have specific consequences with respect to dispersal and the competitive ability of progeny (e.g. Casper 1984, Marshall et al. 1986). Lloyd (1980) postulated that whenever resources for investment in reproduction are unpredictable at early stages of plant development, it is advantageous - with respect to fitness - to regulate maternal investment at the fruiting stage (i.e. in yield components "seeds per ovule" and "single seed weight"). On the other hand, whenever environmental conditions are highly predictable during reproduction, adjustment of production of spikes or flowers is a quicker and more economical means of regulation of maternal investment and should therefore increase the fitness of the parent plant. It is hypothesized that within the former beach-plain population micro-differentiation in patterns of plastic response in seed-yield components occurred due to different selection pressures at the subsites. Plants from the nutrient-poor subsite (a relatively predictable environment) should therefore regulate their investment in reproduction at an early stage of spike development. However, plants from the relatively unpredictable environment of the nutrient-rich subsite should initiate comparatively more spikes and flowers and adjust their maternal investment to the environmental conditions especially at the later fruiting stage. For the purpose of separating micro-environmental and genetic sources of variability inbred lines were reared from plants sampled all over the study site. The present paper reports on a greenhouse experiment on reproductive characteristics of these inbred lines with supply of nutrients as experimental factor.

STUDY SITES

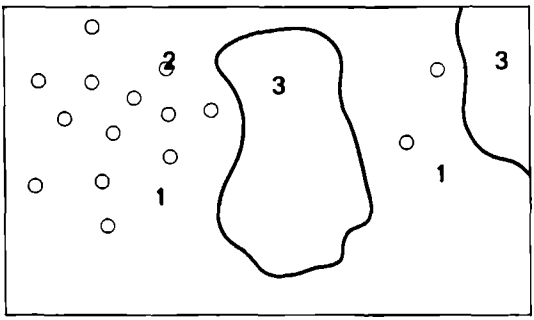
The investigated population of *P. major* ssp. *pleiosperma* is located near

Oostvoornse Meer, a brackish lake along the south west coast of the Netherlands. The Oostvoornse Meer site is a rabbit-grazed former beach plain, embanked in 1966. Because of differences in microrelief, nutrient availability, and vegetation structure a mosaic environment with three subsites can be distinguished (Fig. 1, Table 1). Subsite 1 is a low area whose soil is low in total nitrogen. In winter this area is frequently inundated by rain water. The vegetation is species rich. Cover of higher plant is low (30%). Seed production by *P. major* ssp. *pleiosperma* is very low. Subsite 2 consists of small elevations (0.07-0.15 m high), which are mainly covered with a short dense grassy vegetation. The soil of these patches is relatively high in organic matter and total nitrogen. At this subsite plants of *P. major* ssp. *pleiosperma* produce more seeds than in subsite 1. Subsite 3 has large shrubs of *Hippophae rhamnoides* L. The amount of total nitrogen in the soil is relatively low. But, relative mineralization rates, $NO3^-$-N levels (expressed as percentage of the amount of total nitrogen) of soil samples incubated at 30 °C, were high compared to the other subsites. Around these shrubs, though the standing crop of the herbage layer is relatively high, superficially rooting plants may suffer

Table 1 Estimates of some habitat characteristics, survival rates, and measurements (means ± S D , n = 10) of reproduction of P major ssp pleiosperma at the study site Oostvoornse Meer Three subsites were distinguished (see Fig 1) Data were summarized from Troelstra et al (chapter 5) and Lotz et al (chapter 6)

	Subsite 1	Subsite 2	Subsite 3
Soil (0-10cm)			
organic matter (%)	1 3	2 7	1 4
total N (g m^{-2})	66 4	117 6	68 7
$NO3^-$-N after 20 weeks incubation at 30oC			
(g m^{-2})	7 8	17 1	11 9
(% total N)	11 7	14 5	17 3
Standing crop herbs and grasses (g m^{-2})	14 7	95 2	224 4
Percentage of seedlings emerged in 1985 that survived until October	77	17	1
Reproductive output Plantago seeds (mg $plant^{-1}$)	5 4± 4 2	76 5±63 9	162 8±192 3

Fig. 1. Sketch of the mosaic environment of the Oostvoornse Meer site Three subsites are distinguished. Subsite 1, low area with very open grassland, subsite 2, patches (0.5-1.5 m in diameter) with a higher plant cover of mainly grasses, subsite 3, patches (20-40m in diameter) with shrubs of Hippophaë rhamnoides L



from drought during summer. In this subsite survival rates of *P. major* ssp. *pleiosperma* are low, but plants surviving until the reproductive phase produce large quantities of seeds (Table 1).

METHODS

In each subsite six plants were sampled and self-pollinated to produce inbred lines (two generations). On July 25, 1986 seeds of the six inbred lines from each origin (subsite) were sown in containers with moist dune sand in a greenhouse (20°C). Percentages of germination were at least 83 %. On day 18, after sowing, seedlings were transplanted into pots (contents 0.42 l, one plant per pot) with silver-sand. At the start of the experiment two different nutrient levels were created using a solution twice as strong as described by Steiner (1968) and, with respect to micro-nutrients, as described by Smakman and Hofstra (1982). In the L(ow) treatment 2.6 ml of Steiner solution was put into one pot, in the H(igh) treatment 25 ml. Previous experiments (unpublished data) justified the expectations that these nutrient levels should cause the same range in biomass accumulation as can be found in the study site.

Soil moisture was kept at 20 per cent by weight. Pots were placed in a greenhouse (20°C). During the experiment the natural light period was

extended by artificial lighting to 16 h a day. The number of replicates (one plant per replicate) per inbred line per treatment was five. In order to maintain differences in treatment level during the experiment, uptake of minerals by the plants was compensated by monthly dose of Steiner solution, 2.6 ml in the low treatment pots and 50 ml in the high treatment pots. The plants were harvested when the production of new spikes had almost ended, which was 117 days (treatment H) and 174 days (treatment L) after sowing, respectively. Values of seven seed yield components were determined for each plant: the number of initiated spikes, the number of fructiferous spikes per initiated spike, the number of flowers per fructiferous spike, the number of capsules per flower, the number of ovules per capsule, number of seeds per ovule, and single seed weight. The number of ovules was counted in one developing capsule, sampled from a flower with extended style, at the third spike of a plant. The proportion of ovules which developed into seeds was determined on the basis of the total number of capsules of the same spike. In addition, dry weight of shoots, roots, spikes and total number of seeds of each plant were measured. Data were statistically analyzed using S.P.S.S. subprogramme MANOVA (Nie and Hull 1981). Independent variables were nutrient treatment, origin (subsite), and inbred line nested within origin. Effects of line and nutrients x line were tested against the within-cells Mean Sqares (MS). The effect of origin was tested against the MS determined by line. Effects of nutrients and nutrients x origin were tested against the interaction nutrient x line. If the denominator MS-values of line and nutrients x line were not significant, they were pooled with MS of within-cells. Group means were compared using Duncan 's multiple range tests.

RESULTS

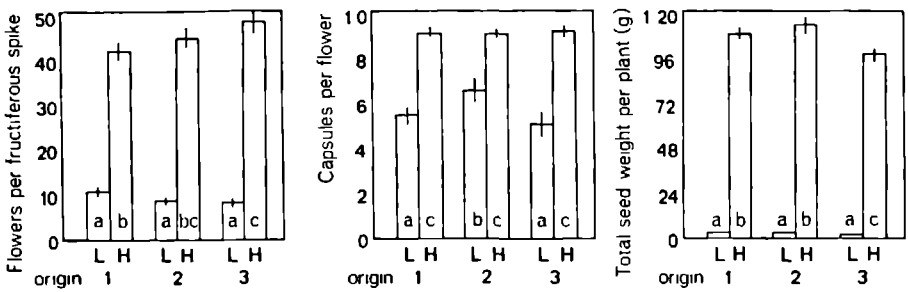
The statistical analysis of the effects of mineral nutrient supply on reproductive characteristics of inbred lines is summarized in Table 2. Most of the seed-yield components were strongly influenced by the nutrient level, having higher values at the high nutrient level. Effects of the nutrient supply on the number of fructiferous spikes per initiated spike and single seed weight, however, were not present. The factor origin (the subsite from which the inbred lines originated) explained variance of only one seed-yield component, the number of capsules per flower. This latter component and the number of flowers per fructiferous spike had both variances explained by nutrient x origin interaction. These interaction

Table 2. Analysis of variance on reproductive characteristics of inbred lines reared from a subdivided population of P. major ssp. pleiosperma. Plants were grown in a greenhouse with two nutrient levels. Inbred lines were nested within origin (subsite). F-values are given, together with degrees of freedom (in parentheses).

	Source of variation				
Dependent variables	Line	Nutrients	Nutrients X Line	Origin	Nutrients X Origin
initiated spikes	3.0(15,128)***	416.3(1,143)***	1.5(15,128)ns	0.1(2, 15)ns	0.4(2,143)ns
fructiferous spikes per initiated spike	1.1(15,127)ns	0.9(1,142)ns	0.9(15,127)ns	2.6(2,142)ns	0.0(2,142)ns
flowers per fructiferous spike	1.9(15,122)*	717.2(1,137)***	1.1(15,122)ns	0.5(2, 15)ns	3.3(2,137)*
capsules per flower	1.0(15,122)ns	170.8(1,137)***	1.3(15,122)ns	4.9(2,137)**	6.4(2,137)**
ovules per capsule	1.6(15,141)ns	317.5(1,156)***	1.5(15,141)ns	0.5(2,156)ns	0.1(2,156)ns
seeds per ovule	3.2(15,103)***	11.2(1, 14)**	2.7(14,103)**	1.9(2, 15)ns	1.7(2, 14)ns
single seed weight	0.7(15,103)ns	0.6(1,118)ns	1.5(15,103)ns	1.3(2,118)ns	0.0(1,118)ns
total seed weight	1.5(15,112)ns	1952.9(1,127)***	1.2(15,112)ns	5.2(2,127)**	2.8(2,127)ns
dry weight spikes/ dry weight plant	1.9(15,113)*	1997.0(1,128)***	0.9(15,113)ns	1.7(2, 15)ns	0.4(2,128)ns

Probability symbols: ns not significant, * P<0.05, ** P<0.01, *** P<0.001.

Fig 2 The effect of the mineral level in silversand on some reproductive characteristics of plants from a subdivided population of P major ssp pleiosperma Data of inbred lines were pooled per origin (subsite) Origin means (±SE, n≥20) are given L Low, 2 6 ml, H High, 75 ml Steiner solution added, Bars, sharing the same letter, indicate means which are not significantly different in Duncan's multiple range tests (P>0 05)



terms indicate genetic variation for phenotypic plasticity between inbred lines of different origins. Subsequent analysis showed that plants of origin 3 showed higher levels of plasticity in number of flowers per fructiferous spike than plants of origin 1 ($P<0.05$, Fig. 2). Besides, at the low nutrient treatment plants of origin 2 had higher numbers of capsules per flower than plants of origin 1 and origin 3 ($P<0.05$). Variance of the total seed weight per plant (measured by weighing isolated seeds) was also partly explained by origin. Plants of origin 3 produced lower seed dry weight at the high nutrient treatment ($P<0.05$). Contrary to this, an origin effect on the reproductive effort (computed as dry weight of all initiated spikes per plant dry weight) was not present. Line effects, representing genetic variation which is not related to subsites, were present for three seed-yield components (initiated spikes, flowers per fructiferous spike, and seeds per ovule) and for the reproductive effort.

DISCUSSION

The present study on *P. major* ssp. *pleiosperma* provides evidence of genetic variability in some reproductive characteristics which are related to local variation in environmental factors such as soil fertility and vegetation

structure. In the greenhouse dry weights of seeds of plants originating from the *Hippophae* subsite was lower than that of plants originating from the surrounding grassland. The absence of differences between origins in plant dry weight (data not presented) and reproductive effort, computed as the total dry weight of spikes per plant dry weight, suggests that under the given experimental conditions plants originating from the *Hippophae* subsite are less efficient in producing seeds.

The mechanism of seed-yield regulation was studied on seven seed-yield components. Five components varied under the different nutrient treatments, demonstrating adjustment of investment in reproduction at almost all stages of development of spikes, flowers, and seeds. No treatment effects, however, were present for the two components "number of fructiferous spikes per initiated spike" and "single seed weight". Marshall et al. (1986) suggested that those yield components, altering of which has relatively strong negative consequences for the fitness of a plant, should be most tightly buffered by performing plasticity in other yield components. In *P. major* abortion of fructiferous spikes may strongly affect fitness because it may imply a relatively large loss of maternal investment in organs that support reproduction (e.g. scapes). Similarly, single seed weight may influence seedling establishment in this beach-plain area.

Plastic responses of two yield components varied between plants originating from different subsites. Plants from subsite 3 showed higher levels of plasticity in number of flowers per fructiferous spike than plants from subsite 1. At the high nutrient level these plants initiated more flowers, but they produced smaller total amounts of seeds. This result supports the hypothesis postulated before. Plants from this subsite with unpredictably fluctuating growth conditions adjust their maternal investment to the availability of resources relatively stronger at later stages of the sequence of yield components. In the natural habitat such pattern of plasticity should allow nurturing of an extra number of seeds when growth conditions improve during the reproductive phase of a plant (Lloyd 1980). It is remarkable that this excess of initiated flowers is only demonstrated at the high nutrient level. Moreover, differences in plasticity in the number of capsules per flower were found between plants originating from subsite 1 and from subsite 2. It is these two subsites which together form a fine-scale mosaic environment with spatial variation over distances of 1 meter. Such small-scale genetic variability suggests low gene flows and high selection pressures.

From the present greenhouse experiment it can not be demonstrated which different selection pressures affected these plasticity patterns. Besides, it is uncertain whether these differences in plasticity simply represent different morphological constraints (Watson and Casper 1984).

Therefore, data are required on gene flow as well as on vegetative and generative characteristics collected in the natural micro-habitats.

The Oostvoornse Meer beach plain was embanked in 1966. For that reason the genetic structure within the *P. major* ssp. *pleiosperma* population should have become established within 20 years. This period of time is in order of magnitude as was found in the Park Grass experiment at Rothamsted, which provided evidence of micro-differentiation to soil factors evolved within 40-60 years (Snaydon 1970, Snaydon and Davies 1972).

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CHAPTER 9
GENERAL DISCUSSION

Each chapter of this thesis has been presented with a discussion of the results. In this final chapter some general aspects of variation in life-history characteristics of *Plantago major* are discussed in relation to environmental variability. Furthermore, some implications of the present results will be discussed in relation to research at the level of whole ecosystems and applied ecology.

Phenotypic plasticity and environment: specificity in both life-history characteristics and environmental factors

As was *a priori* expected (chapter 1), the total phenotypic variation in the life-history characteristics, between the selected populations of *P. major*, was rather large. For example, single seed weight varied 1.5-2 fold and the number of seeds at first reproduction 100-1000 fold. Several authors, e.g. Harper (1977), reported similar ranges in values of life-history characteristics among populations of grassland species. Remarkably, for some life-history characteristics, e.g. number of seeds at first reproduction, the same order of total phenotypic variation was found between three distinguished subpopulations on a beach plain.

The results of the reciprocal sowing experiment (chapter 3) and the reciprocal transplant experiments (chapters 2, 4 and 6) show that the observed variation in the life-history characteristics seed dormancy, age at first flowering, plant size, single seed weight and the number of seeds produced at first or later flowering is for a large part induced by environmental factors. Several authors (e.g. Antonovics and Primack 1982, Blom 1983, Schmidt and Levin 1985) also reported a relatively large influence of the environment on between-population variability in life-history characteristics of naturally occurring grassland species.

It was shown that the variation in life-history characteristics between the populations was also partly due to genetic differences. The genetic variation between populations in germination characteristics, age and size at first flowering and seed production per plant depended on the environment in which the plants were growing, and, therefore, reveal

differences in level of phenotypic plasticity (chapter 2, 3 and 4).

Plant responses to specific environmental factors were studied in detail in a greenhouse. With respect to nutrient supply and inter-specific competition, too, genetic variation in the level of phenotypic plasticity in various life-history characteristics was found between plants from different populations (chapter 2 and 3). Similar results were obtained for other characteristics, e.g. patterns of biomass allocation. In contrast, with respect to variability in nutrient supply and water content of the substrate no differences in phenotypic plasticity in allocation patterns or size related characteristics could be demonstrated between plants from the subsites distinguished within one population (chapter 7). In addition, though over the range of nutrient supplies studied in the greenhouse (chapter 2) a relatively large amount of phenotypic plasticity in single seed weight was demonstrated, no between-population differences in plasticity in this trait were found.

The results demonstrate that in *P. major* there is marked variability in the level of phenotypic plasticity between the life-history characteristics that were investigated and that these plastic responses generally depend on the range of environments or selected environmental factors. This conclusion is in accordance with the observation by Swaegerle and Bazzaz (1987) that plastic responses in various characteristics of *Phlox drummondii* plants with respect to e.g. light regime, were generally independent from the responses to other environmental factors, e.g. nutrient supply. In addition, the present results suggest that the observed genetic variation in several life-history characteristics between populations of *P. major* are mainly based on genetic differences in plastic responses, instead of genetic differences that are expressed independent of the environment.

Variation in life-history characteristics as result of specific selection regimes

As discussed by Endler (1986), hardly any real proof can be obtained that some genetic structure, observed in the field, is the result of natural selection. This proof should at least be based on data on past selection regimes (selection differentials), on shifts in genetic structure in time, and data on gene flow and genetic drift. It is very time consuming to collect these data sets for higher plants. In this section the evidence is discussed on account of which observed genetic variation *between* populations in age at first flowering, seed size and level of plasticity in pattern of biomass allocation might be explained by differences in selection regimes.

Genetic variation between populations in age and size at first flowering

was demonstrated both in greenhouse experiments and at the field sites (chapter 2, 3 and 4). At the river bank early flowering (at least in the first season) was undoubtedly coupled with a high fitness, because due to floods winter mortality was extremely high at this site (chapter 4). Indeed, when the availability of soil nutrients was high enough, plants from the river bank flowered earlier than plants from e.g. the path. An analysis of contemporary selection revealed that at the latter site, plants that initiated flowering relatively early and that had a lower shoot size at that time, had less chance of reaching a second reproduction, than plants that initiated flowering relatively late. At a short distance from the study site on the river bank, *P. major* was found on paths and in meadows. In fact, *P. major* occurs on a continuous gradient from the relatively low edge of the river Rhine (with a short growing season) to sites that are flooded only rarely or not all (Van Dijk 1985, pers. observations). Assuming that the selection regimes at the river bank and the path have not changed in the last decades, it is very likely that the present genetic differences in precocity are caused and maintained by these regimes.

Genetic variation in seed size, too, was found between the investigated populations (chapter 2 and 3). Seeds from the shore-meadow population were, on average, larger than those from the river-bank population. In fluctuating environments (such as the river bank) a plant's fitness may depend more on the number of offspring that escape from unfavourable growth conditions than on seed size and, thus, growth potential of individual seedlings (see for references chapter 3). To explain differences in single seed weight as a result of different selection regimes, at least a cost (in terms of fitness) of producing relatively small seeds needs to be demonstrated at the shore meadow. From the results of a reciprocal transplant experiment and a greenhouse experiment it was hypothesized that such a cost was associated with the germination pattern of the seeds and not with the competitive ability of the seedlings (chapter 3). At this site, mortality of plants until first flowering was extremely high in more or less intact vegetation (chapter 4), compared to that of plants that had emerged in gaps in the vegetation (chapter 3). The ability, associated with a relatively large seed size, to germinate as soon as a gap occurred, e.g. by treading of cattle, will probably be under strong selective pressure at the shore meadow.

In general, *P. major* occurs predominantly in open types of vegetation (Adema 1962, Mólgaard 1976, Haeck et al. 1982). This open vegetation is usually caused by trampling, flooding or other factors which are present for only a relatively short period of time or at a relatively small place. At specific sites where the vegetation recovers from such "setbacks", seed production of *P. major* is relatively high compared to the mean values over the total habitat. To maximize the production of seeds at sites where the

vegetation is recovering, individual plants need to adjust various morphological and physiological processes, e.g. pattern of biomass allocation, to variable growth conditions (Bradshaw 1965, Caswell 1983). Plants from the river bank showed a high degree of phenotypic plasticity in reproductive effort with respect to nutrient supply, whereas plants from the beach plain and the shore meadow, showed a high degree of plasticity in shoot-root ratio (chapter 2). These results suggested that a high degree of phenotypic plasticity in the pattern of biomass distribution over shoot and roots may be an adaptive response to selective forces related to interspecific competition. Namely, by changing the shoot-root ratio plants may adjust their ability to compete either for light or for various nutrients (Brouwer 1963, 1983, Ennik and Baan Hofman 1983). However, from the results of an experiment on competition between *P. major* and *Festuca rubra* it was concluded that plants from the river bank and from the shore meadow did not differ in phenotypic plasticity in leaf-weight ratio with respect to level of interspecific competition (chapter 3). The marked differences in shoot-root ratio between plants from the river bank on the one hand and from the beach plain and the shore meadow on the other (chapter 2), might, however, be explained solely by different selection regimes with respect to levels of competition for soil resources. When soil resources are not limited, plants may maximize their relative growth rate and probably their fitness by allocating a relatively high amount of biomass to the leaves (Brouwer 1963, 1983, Van Noordwijk and De Willigen 1987, chapter 3). Plants, from sites where *P. major* has a perennial life-cycle, increased markedly their biomass allocation to the leaves when the nutrient supply was higher. In this way they consolidated their pre-reproductive development, whereas plants from the river bank (annual life cycle) mainly increased their reproductive output at higher nutrient supply (chapter 2, Fig. 1, Table 2). The differences in habitat characteristics between site Angeren (short growing season) and site Oostvoornse Meer and Kwade Hoek (long life cycle at low soil fertility and high level of interspecific competition, respectively) makes it plausible that the observed differences in phenotypic plasticity in patterns of biomass allocation are to be explained by these different selection regimes (chapter 4).

Comparing plastic responses of species, Crick and Grime (1987), too, related differences in phenotypic plasticity in root-shoot ratio and root morphology to characteristics of the original habitat. *Agrostis stolonifera*, a species from nutrient-rich soil, could considerably better adjust growth and morphology of roots to an increase of nutrient availability than *Scirpus sylvaticus*, a species from infertile soils. These authors argued that the high plasticity observed in *A. stolonifera* allowed the dynamic exploitation of fertile soils by competing roots and predicted that the lower plasticity in *S.*

sylvaticus will confer a selective advantage where nutrients are strongly limiting and only become available in unpredictable, short-lasting pulses. In contrast to the latter species, a relatively high level of plasticity in the shoot-root ratio of *P. major* plants might be of adaptive value at the Oostvoornse Meer site (main area low in mineral nutrients), due to a mozaic pattern -relatively stable in time- of spatial heterogeneity in soil fertility at that site (chapter 5, 7).

Van Andel and Vera (1977) studied plastic responses in reproductive allocation of the annual species *Senecio sylvaticus* and the perennial *Chamaenerion angustifolium*. These authors demonstrated that for both species the reproductive effort of plants that had initiated flowering was almost independent of nutrient supply. However, compared to the annual species, the ratio flowering to non-flowering plants decreased considerably when the nutrient supply was lower. According to these authors, competitive ability of the perennial species was enhanced, due to accumulation of nutrients in the leaf rosette by delay of flowering, whereas the other species, similar to the river-bank plants of *P. major* in the present study, maximized reproductive allocation within their annual life cycle.

Kuiper (1982, 1983) investigated the degree of physiological plasticity of four inbred lines of *P. major*, grown in nutrient solution, after an alteration in the supply of nutrients. Plants of an inbred line from a river bank (subspecies *pleiosperma*) could better adjust their root respiration and ion-stimulated ATP-ase activity, to a change in mineral nutrition, than plants of an inbred line from a lawn (subspecies *major*). Poorter and Lambers (1986) studied growth of these inbred lines in tubes with qartz sand, in which treatments with different frequencies of fluctuations in nutrient level were applied. As the frequency of fluctuations increased, the inbred line from the river bank had a higher competitive ability (i.e. grew faster) than the inbred line from the lawn. These authors suggested that rapid plastic responses in physiological characteristics of the river-bank plants is of advantage in a fluctuating environment where the availablity of nutrients varies throughout the season. If one presumes that short term and local fluctuations in the nutrient availability occur on the river bank and that the nutrient availability is relatively stable at the lawn site, one could probably interprete the differences in physiological characteristics between both lines as the result of different selection regimes. However, at this moment no data set is available that demonstrates that during the growing season soil availability indeed fluctuates more on the river bank than in the soil of the lawn site.

In contrast to the present results for plants from population ANG, both Kuiper (1982, 1983) and Poorter and Lambers (1986) demonstrated a relatively high level of phenotypic plasticity in shoot-root ratio of river-

bank plants with respect to variation in nutrient supply. These authors grew their plants at a relatively short photoperiod (12 h), whereas the present plants were grown at 16 h light. In various herbaceous species induction of flowering and reproductive allocation depends on the length of the photoperiod (Vince-Prue 1986). In *P. major* the number of plants that initiate flowering is considerably increased when the photoperiod is increased from 12 to 16 h (pers. observation). Therefore, the aforementioned differences in plastic response in shoot-root ratio might be the result of differences in growing conditions.

Genetic differentiation in life-history characteristics in relation to scale of environmental variability

Several authors reported small-scale genetic differentiation in various plant characteristics within populations (Linhart and Baker 1973, Snaydon 1970, Snaydon and Davies 1972, Turkington and Aarssen 1984, and references cited there).

Since the open characteristic of the vegetation at the beach plain is merely caused by the fact that the main area has a low soil fertility (chapter 5), differences in habitat characteristics between the different subsites will be relatively stable in time, compared to sites where the open characteristic is caused by biotic factors, e.g. trampling. This fact is an advantage in studying variability in life-history characteristics, because the subsites are relatively easily defined in both space and time.

It is suggested that spatial variability in vegetation structure caused genetic differentiation in allocation pattern, leaf form and flowering phenology within the beach-plain population (chapter 7). These genetic differences in plant characteristics were demonstrated over distances of about 15-25 m (average distance between the low area with the small patches and the shrubs). Plant responses of lines originating from the small patches were generaly intermediate to those from the low area and the shrubs (chapter 6). However, with respect to level of phenotypic plasticity in seed-yield components in response to nutrient supply, even genetic differences were found between plants from the low area and the grassy patches, thus over distances of 0.5-3 m (chapter 8).

In chapters 6-8 various results have been discussed which suggest that the observed genetic differences are the result of different selection regimes. However, to understand the relationship between genetic variation in life-history characteristics (or in level of phenotypic plasticity in these traits) and small-scale variability in selection regimes, data on both selection and gene flow are needed (e.g. Levin et al. 1984, Slatkin 1985). Various

attempts were carried out to estimate the level of (components of) gene flow or the outcrossing rate within the beach-plain population. For example, variation in allozymes was determined to estimate gene flow and outcrossing rates (cf. Van Dijk 1985, Wolff 1988). Because allozyme variation within this specific population appeared to be extremely small, no such estimate could be otained from these data (Wolff 1988). Since this species is a wind-pollinator with a high self-fertilization rate (Van Dijk 1985), special attention was paid to determine the dispersal of seeds. Seed dispersal was estimated by marking seeds in the spike with bright paints and trapping these seeds in the neighbourhood of the plant. However, seeds of *P. major* appeared to be too small for this method (cf. Schaal 1980). Moreover, factors that are rather unpredictable in time, such as floods, tempests, as well as rabbit grazing (chapter 7), may considerably influence seed transport and the post-dispersal fate of the seeds. Trapping of seeds during a specific period of time and within a specific range of distances of a plant, might therefore dramatically underestimate this component of gene flow. As a result, no satisfactory estimate of gene flow within this population was obtained. Apart from contamination problems, a fruitful method to estimate gene flow in an environment such as this beach plain will possibly be the labelling of pollen or seeds, still connected to the plant, with radionuclides and detection of the labels in the next season (Primack and Levy 1988, Winn 1989).

Variation in life-history characteristics: implications to the fields of ecosystem research and applied ecology

Several attempts have been made to explain patterns in plant community structure, e.g. species composition, by processes such as establishment, competition, resource acquisition and succession (e.g. Grime 1979, Van der Maarel 1984, Tilman 1988, and references cited there). These processes are generally characterized with respect to whole species or even to associations of species and no attention is paid to genetic variability within species in life-history characteristics and in the level of phenotypic plasticity in these traits. Ignoring this within-species variability might be reasonable for the aim of simplicity, because at the community level the numerous interactions between biotic and abiotic factors are very complex. Of course, the part of a natural community that is not considered in a simulation analysis of such a system, depends on the requirement that a model must remain easy to handle and lucid (De Wit and Goudriaan 1974). However, with respect to processes in a community system that can be explained on the level of plant responses to environmental factors, a

comparison of performances of models with or without inclusion of within-species variability in life-history characteristics, or related traits, might supply important new insight in the development of plant communities (e.g. Blom 1987). In the mechanistic dynamic model ALLOCATE Tilman (1988) explains large-scale (e.g. landscapes) spatial and temporal variability in vegetation in terms of different abilities to tolerate shortages in particular resources. This author admits that the exclusion from this model of the ability of individual plants to respond plastically to environmental factors might influence model performances. He argues that the impact of plasticity in morphological and physiological traits on simulation results will be low, as the range of such plasticity is small compared to the range of variation among different species. However, Tilman states that there is clearly a need for more empirical data on the range and environmental correlates of plant plasticity, before we will be able to determine the effects of phenotypic plasticity on the structure and dynamics of plant communities. The present thesis clearly satisfies this demand with regard to a grassland species. Though not mentioned by Tilman, a similar statement is valid with respect to rates of genetic differentiation in plant characteristics within a population. In the model ALLOCATE Tilman simulates the results of competition and vegetation development with runs over at least 100 years. From the present results on *P. major* it is concluded that within this period of time genetic differentiation in e.g. patterns of biomass allocation and time of first flowering -both characterics that are incorporated in ALLOCATE to simulate competition- might occur within a population, and this differentiation therefore possibly affects simulated rates of succession.

In weed science, data on life histories and population dynamics are required to improve programmes of weed control. Improvement may be realized by both an increase of the effect of the specific control practices and a reduction of the contamination of the environment, e.g. by herbicide residues. Particularly with respect to the occurrence of genetic differentiation, resulting from selection pressures imposed by standard agricultural practices, e.g. cultivation methods, fertilization regimes, herbicides and crop characteristics, there has been relatively little experimental work on the population biology and evolution of agricultural weeds (Barrett 1988). Data on life-history characteristics and plasticity in these traits, can be used to model the dynamics of weed populations and to enable predictions of changes in population size and growth. Such predictions are relevant to the planning of nature and frequency of particular weed control practices. One has also to decide in this field of applied ecology whether or not to include phenotypic plasticity and genetic variation in life-history characteristics. Incorporation of these features in dynamic simulation studies will increase the realism of the models, but will

seriously complicate the model structure and the effort to collect experimental data to parameterize and validate them.

REFERENCES

Abbott JL (1978) Importance of the organic phosphorus fraction in extracts of calcareous soil. Soil Sci Soc Am J 42:81-85

Adema F (1969) Wat is "*Plantago intermedia*?. Gorteria 12:212-216

Antonovics J, Primack RB (1982) Experimental ecological genetics in *Plantago* VI. The demography of seedling transplants of *P. lanceolata*. J Ecol 70:55-75

Arnold SJ, Wade MJ (1984) On the measurement of natural and sexual selection: theory. Evolution 38:709-719

Austin MP, Austin BO (1980) Behaviour of experimental plant communities along a nutrient gradient. J Ecol 68:891-918

Ball DF, Williams WM (1968) Variability of soil chemical properties in two uncultivated brown earths. J Soil Sci 19:379-391

Barrett SCH (1988) Genetics and evolution of agricultural weeds. In: Altieri MA, Liebmann M (eds) Weed management in agroecosystems: Ecological approaches. CRC Press, Boca Raton, Florida, pp 57-75

Björkman O (1981) Photosynthetic responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Encyclopedia of plant physiology, vol 12A. Springer-Verlag, Berlin, pp 57-107

Björkman O, Holmgren P (1963) Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats. Physiol Plant 16:889-914

Blom CWPM (1978) Germination, seedling emergence and establishment of some *Plantago* species under laboratory and field conditions. Acta Bot Neerl 27:257-271

Blom CWPM (1979) Effects of trampling and soil compaction on the occurrence of some *Plantago* species in coastal sand dunes. Thesis, University of Nijmegen, the Netherlands

Blom CWPM (1983) Plasticity of life characteristics in two different populations of *Plantago maritima*. Oecol Plant 4:377-394

Blom CWPM (1987) Experimental plant ecology as an approach in coastal population biology. In: Huiskes AHL, Blom CWPM and Rozema J (eds) Vegetation between land and sea. Junk Publishers, Dordrecht, pp 312-325.

Blom CWPM (1988) The realism of models in plant demography. Acta

Bot Neerl 37:421-438
Blom CWPM, Lotz LAP (1985) Phenotypic plasticity and genetic differentiation of demographic characteristics in some *Plantago* species. In: Haeck J, Woldendorp JW (eds) Structure and functioning of plant populations/ 2. Phenotypic and genotypic variation in plant populations. North-Holland Publishing Company, Amsterdam
Bowman RA, Cole CV (1978a) Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. Soil Sci 125:49-54
Bowman RA, Cole CV (1978b) An exploratory method for fractionation of organic phosphorus from grassland soils. Soil Sci 125:95-101
Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115-155
Bradshaw AD (1972) Some of the evolutionary consequences of being a plant. Evol Bol 5:25-47
Brouwer R (1962) Nutritive influences on the distribution of dry matter in the plant. Neth J agric Sci 10:399-408
Brouwer R (1963) Some aspects of the equilibrium between overground and underground parts. Jaarboek IBS Wageningen 1963:31-39
Carson HL, Templeton AR (1984) Genetic revolutions in relation to speciation phenomena: the founding of new populations. Ann Rev Syst 15:97-131
Casper BA (1984) On the evolution of embryo abortion in the herbaceous perennial *Crytantha flava*. Evolution 38:1337-1349
Caswell H (1983) Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences. Am Zool 23:35-46
Chapin FS III (1980) The mineral nutrition of wild plants. Ann Rev Ecol Syst 11:233-260
Chapin FS, Vitousek PM, Van Cleve K (1986) The nature of nutrient limitation in plant communities. Am Nat 127:48-58
Charlesworth B, Leon JA (1976) The relation of reproductive effort to age. Am Nat 110:449-459
Choe HS, Chu C, Koch G, Gorham J, Mooney HA (1988) Seed weight and seed resources in relation to plant growth rate. Oecologia (Berlin) 76:158-159
Cohen D (1966) Optimizing reproduction in a randomly varying environment. J Theoret Biol 12:119-129
Cole CV, Innis GS, Stewart JWB (1977) Simulation of phosphorus cycling in semi-arid grasslands. Ecology 58:1-15

Cole LC (1954) The population consequences of life history phenomena. Quart Rev Bol 29:103-137
Cook R (1980) The biology of seeds in the soil. In: Solbrig OT (ed) Demography and evolution in plant populations. Blackwell, Oxford, pp 107-129
Crick JC, Grime JP (1987) Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. New Phytol 107:403-414
Davies BE (1974) Loss-on-ignition as an estimate of soil organic matter. Soil Sci Soc Amer Proc 38:150-151
Davy AJ, Taylor K (1974) Seasonal patterns of nitrogen availability in contrasting soils in the Chiltern Hills. J Ecol 62:793-807
De Jager A, Posno M (1979) A comparison of the reaction to a localized supply of phosphate in *Plantago major*, *Plantago lanceolata* and *Plantago media*. Acta Bot Neerl 28:479-489
De Jong TJ, Klinkhamer PGL (1987) *Cynoglossum*. In: Halevy AH (ed) Handbook of flowering VI, CRC press, Florida
De Wit CT, Goudriaan J (1978) Simulation of ecological processes. PUDOC, Wageningen, the Netherlands
Dirzo R, Sarukhan J (1984) Perspectives on plant population ecology. Sinauer, Sunderland
Dijkstra P (in press) Cause and effect of differences in specific leaf area. In: Lambers H, Camebridge ML, Koning H, Pons TL (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic Publishing, The Hague
Dijkstra P, Lambers H (1986) Photosynthesis and respiration of two inbred lines of *Plantago major* L. differing in relative growth rate. In: Marcell R, Clijsters H, Poucke M (eds) Biological control of photosynthesis. Martinus Nijhoff Publishers, The Hague
Dijkstra P, Lambers H (in press) A physiological analysis of genetic variation in relative growth rate within *Plantago major*. Fuctional Ecology 3:xx-xx
Dixon WJ (1985) BMDP Statistical Software. University of California Press. Berkely
Endler JA (1986) Natural selection in the wild. Monographs in Population Biology Vol. 21. Princeton Univ Press, Princeton
Ennik GC, Baan Hofman T (1983) Variation in the root mass of ryegrass types and its ecological consequences. Neth J agric Sci 31:325-334
Falconer DS (1981) Introduction to quantitative genetics. 2nd edn, Longman, London

Fenner M (1978) A comparison of the abilities of colonizers and closed-turf species to establish from seed in artificial swards. J Ecol 66:953-963
Fenner M (1983) Relationships between seed weight, ash content and seedling growth in twenty-four species of Compositae. New Phytol 95:697-706
Fenner M (1987) Seedlings. New Phytol 106 (Suppl):35-47
Fowler NL, Antonovics J (1981) Small-scale variability in the demography of transplants of two herbaceous species. Ecology 62:1450-1457
Friesen DK, Blair GJ, Duncan M (1985) Temporal fluctuations in soil test values under permanent pasture in New England, NSW. Aust J Soil Res 23:181-193
Gibson DJ (1988) The maintenance of plant and soil heterogeneity in dune grassland. J Ecol 76:497-508
Givnish TJ (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol 106:131-160
Grime JP (1979) Plant strategies and vegetation processes. John Wiley & Sons, Chichester
Gross KL (1984) Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. J Ecol 72:369-387
Gupta PL, Rorison IH (1975) Seasonal differences in the availability of nutrients down a podzolic profile. J Ecol 63:521-534
Haeck J, Van der Aart PJM, Dorenbosch H, Van der Maarel E, Van Tongeren O (1982) The occurrence of *Plantago* species in ordinated Dutch plant communities. Verh Kon Ned Akad Wetensch, afd Natuurk II, 79 Progress Report IOO 1981:26-33
Haeck J, Woldendorp JW (1985) Structure and functioning of plant populations/ 2. Phenotypic and genotypic variation in plant populations. North-Holland Publishing Company, Amsterdam
Haines SG, Cleveland G (1981) Seasonal variation in properties of five forest soils in southwest Georgia. Soil Sci Soc Am 45:139-143
Halm BJ, Stewart JWB, Halstead RL (1972) The phosphorus cycle in a native grassland ecosystem. In: Isotopes and Radiation in Soil-Plant Relationships including Forestry. Proc Symp IAEA:571-586
Harper JL (1977) The population biology of plants. Academic Press, London
Harrison AF (1979) Variation of four phosphorus properties in

woodland soils. Soil Biol Biochem 11:393-403
Hartgerink AP, Bazzaz FA (1984) Seedling-scale environmental heterogeneity influences individual fitness and population structure. Ecology 65:198-206
Heathcote CA, Davies MS, Etherington JR (1987) Phenotypic flexibility of *Carex flacca* Schreb. Tolerance of soil flooding by populations from contrasting habitats. New Phytol 105:381-391
Hedrick PW (1986) Genetic polymorphism in heterogeneous environments: a decade later. Ann Rev Ecol Syst 17:535-566
Hickman JC (1975) Environmental unpredictability and plastic energy allocation strategies in annual Polygonum cascadense (Polygonaceae). J Ecol 63:687-701
Hirose T (1988) Modelling the relative growth rate as a function of plant nitrogen concentration. Physiol Plant 72:185-189
Hirose T, Kachi N (1986) Graphical analysis of the life history evolution of *Oenothera glazioviana* Micheli. Oecologia (Berlin) 68:490-495
Jain S (1979) Adaptive strategies: Polymorphism, plasticity, and homeostasis. In: Solbrig OT, Jain S, Johnson GB, Raven PH (eds) Topics in plant population biology. Blackwell, Oxford, pp 160-187
Jones R, Etherington JR (1971) Comparative studies of plant growth and distribution in relation to waterlogging IV. The growth of dune and dune slack plants. J Ecol 59:793-801
Kachi N, Hirose T (1983) Limiting nutrients for plant growth in coastal sand dune soils. J Ecol 71:937-944
Keeney DR, Nelson DW (1982) Nitrogen - inorganic forms. In: Page AL, Miller RH, Keeney DR (Eds) Methods of Soil Analysis, Part 2 (2nd edition). Agronomy 9:643-698
Kempers AJ (1974) Determination of sub-microquantities of ammonium and nitrates in soils with phenol, sodiumnitroprusside and hypochlorite. Geoderma 12:201-206
Kromer M, Gross KL (1987). Seed mass, genotype, and density effects on growth and yield of *Oenothera biennis* L. Oecologia (Berlin) 73:207-212
Kuiper D (1982) Genetic differentiation in *Plantago major*: Ca^{2+}- and Mg^{2+}-stimulated ATPases from roots and their role in phenotypic adaptation. Physiol Plant 56:436-443
Kuiper D (1983) Genetic differentiation in *Plantago major*: growth and root respiration and their role in phenotypic adaptation. Physiol Plant 57:222-230
Kuiper D (1984) Genetic differentiation and phenotypic plasticity

in *Plantago* species. Thesis, University of Groningen
Lacey EP (1986) The genetic and environmental control of reproductive timing in a short-lived monocarp *Daucus carota* (Umbelliferae). J Ecol 74:73-86
Lacey EP, Real L, Antonovics J, Heckel DG (1983) Variance models in the study of life histories. Am Nat 122:114-131
Lambers H, Dijkstra P (1987) A physiological analysis of genotypic variation in relative growth rate: Can growth rate confer ecological advantage?. In: Van Andel J, Bakker JP, Snaydon RW (eds) Disturbance in grasslands. Junk Publishers, Dordrecht, the Netherlands
Lande R, Arnold SJ (1983) The measurement of selection on correlated characteristics. Evolution 37:1210-1226
Law R, Bradshaw AD, Putwain PD (1977) Life-history variation in *Poa annua*. Evolution 31:233-246
Levin DA (1984) Immigration in plants: an exercise in the subjunctive. In: Dirzo R, Sarukhan J (eds) Perspectives on plant population ecology. Sinaur, Sunderland, pp 242-260
Levin DA, Cohen D, Hasting A (1984) Dispersal strategies in patchy environments. Theor Pop Biol 26:165-191
Linhart YB, Baker I (1973) Intra-populational differentiation in response to flooding in a population of *Veronica peregrina* L. Nature 242:275-276
Lloyd DG (1980) Sexual strategies in plants 1. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol 86:69-79
Lloyd DG (1984) Variation strategies of plants in heterogeous environments. Biol J Linn Soc 21:357-385
Lloyd DG (1987) Selection of offspring size at independence and other size-versus-number strategies. Am Nat 129:800-817
Lotz LAP (1985) Variation in some traits of life history and morphology between populations of *Plantago major* L. in a garden experiment. Verh Kon Ned Akad Wetensch, afd Natuurk II, 83 Progress Report IOO 1984:45-49
Lotz LAP, Blom CWPM (1986) Plasicity in life-history traits of *Plantago major* L. ssp. *pleiosperma* Pilger. Oecologia (Berlin) 69:25-30 (chapter 2)
Lotz LAP, Olff H (1988) Variation in biomass and architecture of plants due to small-scale environmental heterogeneity. In: Werger MJA, Van der Aart PJM, During HJ, Verhoeven JTA (eds) Plant form and vegetation structure, SPB Academic Publishing, the Hague, the Netherlands, pp 109-119
Lotz LAP, Spoormakers LDH (1988) Differentiation in reproductive

characteristics within a population of *Plantago major* L. ssp. *pleiosperma* Pilger. Oecol Plant 9:11-18 (chapter 8)
Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Ann Rev Syst 15:65-95
Mackay DB (1972) The measurement of viability. In: Roberts EH (ed) Viability of seeds. Chapman and Hall, London, pp 172-208
Marshall DL, Levin DA, Fowler NL (1986) Plasticity of yield components in response to stress in *Sesbania macrocarpa* and *Sesbania vesicaria* (*Leguminosae*). Am Nat 127:508-521
McGarity JW, Myers RJK (1973) Seasonal trends in the content of mineral nitrogen in solodized solonetz soil. Aust J Exp Agric Anim Husb 13:423-429
McGraw JB, Antonovics J (1983) Experimental ecology of *Dryas octopetala* ecotypes. 1. Ecotypic differentiation and life-cycle stages of selection. J Ecol 71:879-897
Mengel K, Kirkby EA (1987) Principles of Plant Nutrition (4th edition). International Potash Institute, Bern
Miller RG (1981) Silmultaneous statistical inference. Second edition. Springer, New York
Mitchell-Olds T, Shaw R (1987) Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149-1161
Mølgaard P (1976) *Plantago major* ssp. *major* and ssp. *pleiosperma*: Morphology, biology and ecology in Denmark. Bot Tidsskr 71:31-56
Naylor REL (1980) Effects of seed size and emergence time on subsequent growth of perennial ryegrass. New Phytol 84:313-318
Nie NH, Hull CH (1981) SPSS update 7-9: New procedures and facilities for releases. McGraw-Hill, New York
Norusis MJ (1986) SPSS/PC + advanced statistics. SPSS inc Chicago
Novozamsky I, Houba VJG, Temminghoff E, Van der Lee JJ (1984) Determination of 'total' N and 'total' P in a single soil digest. Neth J Agric Sci 32:322-324
Nye PH, Tinker PB (1977) Solute movement in the soil-root system. Blackwell Scientific Publications, Oxford
Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ 939
Parrish J, Bazzaz FA (1982) Responses of plants from three successional communities to a nutrient gradient. J Ecol 70:233-248
Patrick Jr WH, Mikkelsen DS (1971) Plant nutrient behavior in

flooded soil. In: Olson RA, Army TJ, Hanway JJ, Kilmer VJ (Eds) Fertilizer Technology and Use (2nd edition): pp 187-215
Peterson DL, Hammer RD (1986) Soil nutrient flux: a component of nutrient cycling in temperate forest ecosystems. For Sci 32:318-324
Peterson DL, Rolfe GL (1982) Seasonal variation in nutrients of flood plain and upland forest soils of central Illinois. Soil Sci Soc Am J 46:1310-1315
Peterson DL, Rolfe GL (1985) Temporal variation in nutrient status of a floodplain soil. For Ecol Manage 12:73-82
Pons TL, Van der Toorn J (1988) Establishment of *Plantago lanceolata* L. and *Plantago major* L. among grass. I. Significance of light for germination. Oecologia (Berlin) 75: 394-399
Poorter H, Lambers H (1986) Growth and competitive ability of a highly plastic and a marginally plastic genotype of *Plantago major* in a fluctuating environment. Physiol Plant 67:217-222
Primack RB (1978) Regulation of seed yield in *Plantago*. J Ecol 66:835-847
Primack RB, Antonovics J (1981) Experimental ecological genetics in *Plantago*. V. Components of seed yield in the ribwort plantain *Plantago lanceolata* L. Evolution 35:1069-1079
Primack RB, Levy CK (1988) A method to label seeds and seedlings using gamma-emitting radionuclides. Ecology 69, 796-800
Pyke DA, Thompson JN (1986) Statistical analysis of survival and removal rate experiments. Ecology 67:240-245
Ray KN, Jain SK (1982) Population Biology of *Avena* IX. Gene flow and neighborhood size in relation to microgeographic variation in *Avena barbata*. Oecologia (Berlin) 53:399-405
Reznick D (1985) Costs of reproduction: an evaluation of the empirical evidence. Oikos 44:257-267
Rice SA, Bazzaz FA (1989) Growth consequences of plasticity of plant traits in response to light conditions. Oecologia 78:508-512
Ridge I (1987) Ethylene and growth control in amphibious plants. In: Crawford RMM (Ed) Plant life in aquatic and amphibious habitats. Blackwell Scientific Pubications, Oxford
Salisbury EJ (1942) The reproductive capacity of plants. Bell, London
Samson DA, Werk KS (1986) Size-dependent effects in the analysis of reproductive effort in plants. Am Nat 127:667-680
Saunders WMH, Metson AJ (1971) Seasonal variation of phosphorus in soil and pasture. N Z J Agric Res 14:307-328

Schaal BA (1980) Measurement of gene flow in *Lupinus texensis*. Nature 284:450-451

Schaal BA (1984) Life-history variation, natural selection, and maternal effects in plant populations. In: Dirzo R, Sarukhan J (eds) Perspectives on plant population ecology. Sinaur, Sunderland, pp 188-206

Schat H (1984) A comparative ecophysiological study on the effects of waterlogging and submergence on dune slack plants: growth, survival and mineral nutrition in sand culture experiments. Oecologia (Berlin) 62:279-286

Scheiner SM, Goodnight CJ (1984) The comparison of phenotypic plasticity and genetic variation in populations of the grass *Danthonia spicata*. Evolution 38:845-855

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Ann Rev Ecol Syst 17:667-693

Schmidt KP, Levin DA (1985) The comparative demography of reciprocally sown populations of *Phlox drummondii* Hook. I. Survivorships, fecundities, and finite rates of increase. Evolution 39:396-404

Schmitt J (1983) Individual flowering phenology, plant size, and reproductive succes in *Linanthus androsaceus*, a California annual. Oecologia (Berlin) 59:135-140

Schwaegerle KE, Bazzaz FA (1987) Differentiation among nine populations of *Phlox*: response to environmental gradients. Ecology 68:54-64

Schwintzer CR, Lancelle SA (1983) Effect of water-table depth on shoot growth, root growth, and nodulation of *Myrica Gale* seedlings. J Ecol 71:489-501

Silander JA (1985) The genetic basis of the ecological amplitude of *Spartina patens*. II. Variance and correlation analysis. Evolution 39: 1034-1052

Silvertown J (1989) The paradox of seed size and adaptation. TREE 4:24-26

Slatkin M (1985) Gene flow in natural populations. Ann Rev Ecol Syst 16:393-430

Smakman G, Hofstra JJ (1982) Energy metabolism of *Plantago lanceolata*, as affected by change in root temperature. Phyiol Plant 56:33-37

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108:499-506

Snaydon RW (1970) Rapid population differentiation in a mosaic environment. I. The response of *Anthoxanthum odoratum* populations to soils. Evolution 24:257-269

Snaydon RW, Davies MS (1972) Rapid population differentiation in a mosaic environment. II. Morphological variation in *Anthoxanthum odoratum*. Evolution 26:390-405
Soekarjo R (1983) Germination of *Plantago major* seeds of different stages of maturity. Verh Kon Ned Akad Wetensch, Afd Natuurk II 81, Progress Report IOO:41-42
Sokal RR, Rohlf F (1981) Biometry. The principles and practice of statistics in biological research. Freeman and Company, New York
Sørensen T (1954) Adaptation of small plants to deficient nutrition and a short growing season, illustrated by cultivation experiments with *Capsella bursa pastoris* (L.) Med. Bot Tidsskr 51:339-361
Soule JD, Werner PA (1981) Patterns of resource allocation in plants, with special reference to *Potentilla recta*. L Bull Torrey Bot Club 108:311-319
Stanton ML (1984) Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. Ecology 65: 1105-1112
Stanton ML (1985) Seed size and emergence time within a stand of wild radish (*Raphanus raphanistrum* L.): the establishment of a fitness hierarchy. Oecologia (Berlin) 67:524-531
Stearns CS (1977) The evolution of life history traits: A critique of the theory and a review of the data. Ann Rev Ecol Syst 8:145-171
Stearns CS, Koella JC (1986) The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40:893-913
Stebbins GL (1971) Adaptive radiation of reproductive characteristics in angiosperms. II: Seeds and seedlings. Ann Rev Ecol Syst 2:237-260
Steiner AA (1968) Soilless culture. Proc 6th Int Potash Inst, Florence, pp 324-341
Schemske DW (1984) Population structure and local selection in *Impatiens pallida* (*Balsaminaceae*), a selfing annual. Evolution 38:817-832
Talbot RJ, Etherington JR, Bryant JA (1987) Comparative studies of plant growth and distribution in relation to waterlogging XII. Growth, photosynthetic capacity and metal ion uptake in *Salix caprea* and *S. cinera* ssp. *oleifolia*. New Phytol 105:563-574
Taylor AA, De-Felice J, Havill DC (1982) Seasonal variation in nitrogen availability and utilization in an acidic and

calcareous soil. New Phytol 92:141-152
Ter Borg SJ (1972) Variability of *Rhinanthus serotinus* (Schönh.) Oborny in relation to environment. Thesis. University of Groningen, the Netherlands.
Thompson K (1987) Seeds and seed banks. New Phytol 106 (Suppl): 23-34
Thompson PA (1981) Variation in seed size within populations of *Silene dioica* (L.) Clairv. in relation to habitat. Ann Bot 47: 623-634
Tilman D (1985) The resource ratio hypothesis of succession. Am Nat 125:827-852
Tilman D (1988) Plant strategies and the dynamics and structure of plant communities. Monographs in population Biology. Princeton University Press, Princeton
Troelstra SR (1983) Growth of *Plantago lanceolata* and *Plantago major* on a NO_3/NH_4 medium and the estimation of the utilization of nitrate and ammonium from ionic-balance aspects. Plant and Soil 76:183-197
Troelstra SR, BlacquiÈre T, Wagenaar R, Van Dijk C (1987) Ionic balance, proton efflux, nitrate reductase activity and growth of *Hippophae rhamnoides* L. ssp *rhamnoides* as influenced by combined-N nutrition or N_2 fixation. Plant Soil 103:169-183
Troelstra SR, Sluimer L, Smant W, Wagenaar R, Van der Meulen MA (1981) On the soil chemistry of natural habitats of *Plantago* species and *Hypochaeris radicata* in various parts of the Netherlands in relation to the chemical composition of the plants. Verh Kon Ned Akad Wetensch, Afd Natuurk II 77, Progress Report IOO:22-32
Troelstra SR, Smant W, Wagenaar R (1983) Different K and P requirements for *Plantago major* ssp. *major* and *Plantago lanceolata*? Verh Kon Ned Akad Wetensch, Afd Natuurk II 81, Progress Report IOO:43-54
Trought MCT, Drew MC (1980) The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.) I. Shoot and root growth in relation to changes in the concentrations of dissolved gases and solutes in the soil solution. Plant and soil 54:77-94
Turkington R, Aarssen LW (1984) Local-scale differentiation as a result of competitive interactions. In: Dirzo R, Sarukhan J (eds) Perspectives on plant population ecology. Sinaur, Sunderland, pp 107-127
Van Andel J, Vera F (1977) Reproductive allocation in *Senecio sylvaticus* and *Chamaenerion angustifolium* in relation to

mineral nutrition. J Ecol 65:747-758
Van Damme JMM (1983) On gynodiocy in *Plantago lanceolata*. Thesis, University of Groningen, the Netherlands
Van der Aart PJM (1985) Demographic, genetic and ecophysiological variation in *Plantago major* and *P. lanceolata* in relation to vegetation type. In: White J (ed) The population structure of vegetation. Junk Publishers, Dordrecht, the Netherlands, pp 441-462
Van der Maarel E (1984) Dynamics of plant populations from a synecological viewpoint. In: Dirzo R, Sarukhan J (eds) Perspectives on plant population ecology. Sinaur, Sunderland, pp 66-82
Van der Toorn J, Pons TL (1988) Establishment of *Plantago lanceolata* L. and *Plantago major* L. among grass. I. Shade tolerance of seedlings and selection on time of germination. Oecologia (Berlin) 76:341-347
Van der Toorn J, Van Tienderen PH, Mook JH (submitted) Variability in morphological and life history traits in *Plantago lanceolata* L. in relation to the environment.
Van Dijk H (1984) Genetic variability in *Plantago* species in relation to their ecology. II. Quantitative characters and allozyme loci in *P. major*. Theoret Appl Genet 68:43-52
Van Dijk H (1985) Genetic variability in *Plantago* species in relation to their ecology. Thesis. University of Groningen, the Netherlands
Van Dijk H, Van Delden W (1981) Genetic variability in *Plantago* species in relation to their ecology, I. Genetic analysis of the allozyme variation in *P. major* subspecies. Theor Appl Genet 60:285-290
Van Groenendael JM (1985) Selection for different life histories in *Plantago lanceolata* L. Thesis, Catholic University, Nijmegen, the Netherlands
Van Groenendael JM (1986) Life history characteristics of two ecotypes of *Plantago lanceolata* L. Acta Bot Neerl 35: 71-86
Van Noordwijk M, De Willigen P (1987) Agricultural concepts of roots: from morphogenetic to functional equilibrium between root and shoot growth. Neth J agric Sci 35:487-496
Van Tienderen PH (1989) On the morphology of *Plantago lanceolata*: selection, adaptation, constraints. Thesis, University of Utrecht, the Netherlands
Van Tienderen PH (in press) Measuring selection on quantitative characters: a discussion of some problems and merits of the quantitative genetic approach, applied to plant populations.

Evolutionary Trends in Plants 3:xx-xx
Vaughn CE, Center DM, Jones MB (1986) Seasonal fluctuations in nutrient availability in some northern California annual range soils. Soil Sci 141:43-51
Venable DL (1984) Using intraspecific variation to study the ecological significance and evolution of plant life-histories. In: Dirzo R, Sarukhan J (eds), Perspectives on plant population ecology. Sinauer, Sunderland, pp 166-187
Venable DL, Lawlor L (1980) Delayed germination and dispersal in desert annuals: escape in space and time. Oecologia (Berlin) 46: 272-282
Veresoglou DS, Fitter AH (1984) Spatial and temporal patterns of growth and nutrient uptake of five co-existing grasses. J Ecol 72:259-272
Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505-512
Vince-Prue D (1986) The duration of light and photoperiodic responses. In: Kendrick RE, Kronenberg GHM (eds) Photomorphogenesis in plants. Martinus Nijhoff Publ, Dordrecht, the Netherlands
Voesenek LACJ, Blom CWPM (1989) Growth responses of *Rumex* species in relation to submergence and ethylene. Plant, Cell and Environment 12:433-439
Warwick SI, Thompson BK, Black LD (1987) Life history and allozyme variation in populations of the weed species *Setaria faberi*. Can J Bot 65:1396-1402
Watanabe FS, Olsen SR (1965) Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci Soc Amer Proc 29:677-678
Watkinson AR, Gibson CC (1985) Life-history variation and the demography of plant populations. In: Haeck J, Woldendorp JW (eds) Structure and function of plant populations/ 2. Phenotypic plasticity and genotypic variation, North-Holland Publishing Company, Amsterdam, pp 105-113
Watson MA, Casper BB (1984) Morhogenetic constraints on patterns of carbon distribution in plants. Ann Rev Ecol Syst 15:233-258
Weis IM (1982) The effect of propagule size on germination and seedling growth in *Mirabilis hirsuta*. Can J Bot 60:1868-1874
Werner PA (1985) Phenotypic variation and implications for reproductive success. In: Haeck J, Woldendorp JW (eds) Structure and functioning of plant populations/ 2. Phenotypic and genotypic variation in plant populations. North-Holland

Publishing Company, Amsterdam, pp 1-26
Wilbur HM (1977) Propagule size, number, and dispersion pattern in *Ambystoma* and *Asclepias*. Am Nat 111:43-68
Wild A (1981) Mass flow and diffusion. In: Greenland DJ, Hyes MHB (Eds) The chemistry of soil processes. J Wiley and Sons. pp 37-80
Williams JT (1969) Mineral nitrogen in British grassland soils. I. Seasonal patterns in simple models. Oecol Plant 4:307-320
Winn AA (1985) The effects of seed size and microsite on seedling emergence in four populations of *Prunella vulgaris*. J Ecol 73: 831-840
Winn AA (1988) Ecological and evolutionary consequences of seed size in *Prunella vulgaris*. Ecology 69:1537-1544
Winn AA (1989) Using radionuclide labels to determine the post-dispersal fate of seeds. TREE 4:1-2
Wolff K (1988) Natural selection in *Plantago* species: a genetical analysis of ecologically relevant morhological variability. Thesis, University of Groningen, the Netherlands
Wulff RD (1973) Intrapopulational variation in the germination of seeds in *Hyptis suaveolens*. Ecology 54:646-649
Wulff RD (1986a) Seed size variation in *Desmodium paniculatum* II. Effects on seedling growth and physiological performance. J Ecol 74:99-114
Wulff RD (1986b) Seed size variation in *Desmodium paniculatum* III. Effects on reproductive yield and competitive ability. J Ecol 74:115-121
Zimmerman JK, Weis IM (1983) Fruit size variation and its effects on germination and seedling growth in *Xanthium strumarium*. Can J Bot 61:2309-2315

SUMMARY

The main aim of the investigations described in this thesis was to relate variation in life-history characteristics of a grassland species, to environmental variability. Knowledge of this relationship is essential in order to understand the adaptation of organisms to their environment. In this respect it is important to question whether variation in life-history characteristics, between and within populations, is based on genetic differentiation or induced by the environment (phenotypic plasticity) and whether differences in these characteristics, or in level of phenotypic plasticity in these traits, may be explained by different selection regimes. This study was performed on *Plantago major* L., because this perennial species occurs in very contrasting habitats, e.g. with respect to structure and nutrient content of the soil, level of interspecific competition, and the presence of environmental factors which cause winter mortality.

In a short-term reciprocal transplant experiment and a greenhouse experiment in which plants were grown at three nutrient levels, it was demonstrated that differences in growth and reproduction between three populations of *P. major* ssp. *pleiosperma* were determined rather by environmental factors than by genetic differentiation (chapter 2). Genetic differences were found, among others, in seed size, age at first flowering and in the level of phenotypic plasticity in the pattern of biomass allocation over vegetative and generative parts of the plant. Plants from a river bank had, on average, smaller seeds, flowered earlier and had a lower level of phenotypic plasticity in reproductive effort than plants from a shore meadow.

Effect of seed size of *P. major* on germination pattern and competitive ability was analysed in a reciprocal sowing experiment and a greenhouse experiment in which *P. major* plants were grown in different densities of *Festuca rubra* L. (chapter 3). Relatively small seeds showed a higher level of dormancy than large seeds. No effect of seed size on competitive ability of seedlings could be demonstrated. It is argued that the observed differences in seed size may be a result of different selection regimes. In the unpredictable river-bank environment, plants will increase fitness by producing many, small seeds. However, in the shore meadow the production of small seeds might involve a cost in terms of fitness which is associated with a long period of dormancy in this environment with a high level of interspecific competition. The ability to germinate as soon as a gap in the dense vegetation has been created will probably be under strong selective pressure.

Variation in age and size at first flowering was further investigated in a reciprocal transplant experiment with plants from five populations (chapter 4). Plants from the river bank, a former beach plain and a dune grassland flowered, on average, one or more years earlier than plants from a path and a shore meadow. These genetic differences corresponded qua direction with selection differentials of age and size that were determined for the reproductive phase. The path plants were large and increased fitness by flowering relatively late, in contrast to the plants from the river bank, the beach plain and the dune grassland. Plants at the path site, that initiated flowering relatively early, when the shoot was still small, had a less chance of reproducing a second time, than plants that initiated flowering relatively late. At the shore meadow survival in the pre-reproductive phase was very low. It was suggested, that at this site, too, plants might increase fitness by flowering late.

Evidence is discussed that the aforementioned between-population differences in patterns of biomass allocation (chapter 2) can also be explained by differences in selection regimes. Plants from the beach plain and the shore meadow (long life cycle at low soil fertility and high level of interspecific competition, respectively) markedly increased their biomass allocation to the leaves at a higher nutrient supply and thus consolidated their pre-reproductive development, whereas plants from the river bank (annual life cycle), increased their reproductive output mostly at higher nutrient supply.

Within-population variability in life-history characteristics was studied in detail at the former beach plain. At this site, embanked in 1966, a mosaic environment was distinguished with spatial variability in nutrient availability and water content of the soil as well as in vegetation structure (chapter 5). Contemporary selection on *P. major* was studied in three subsites (chapter 6). In one subsite, patches (c. 1 m^2) with a high availability of nutrients and a dense cover of grasses, plants with a relatively high number of large leaves (both long and broad) had a higher total seed production than other plants. In the other subsites, an area with a low availability of nutrients and a low cover of higher plants and an area with shrubs of *Hippophae rhamnoides* L., leaf width, and not leaf length, was possitively correlated with relative fitness.

The effects of nutrient supply and waterlogging on morphology and life history were studied on lines from the three subsites in a greenhouse (chapter 7). For most of the traits studied high levels of phenotypic plasticity were observed, covering almost entirely the observed phenotypic variability of *P. major* at the beach plain. However, in all treatments lines from the shrubs had a higher leaf-area ratio as well as delayed flowering when compared to lines from more open subsites. In addition, in a reciprocal transplant experiment it was demonstrated that lines from the

shrubs had larger shoots with e.g. broader leaves in the shady environment of the shrubs than other lines (chapter 6).

From the experiments no indications were obtained that lines from any subsite were specially adapted to specific levels of nutrient supply or water content of the soil. With respect to these environmental factors *P. major* might occur and reproduce in all subsites by means of phenotypic plasticity, e.g. in plant form. However, it is suggested that spatial variability in vegetation structure (i.e. light intensity at the soil surface) caused a population subdivision in allocation patterns, leaf form and life history of *P. major* at the beach plain, over distances of about 15-25 m, during primary succession over a period of twenty years.

In addition, genetic differentiation within the beach-plain population for specific levels of phenotypic plasticity in seed-yield components was studied in a greenhouse experiment in which the plants were grown at two levels of nutrient supply (chapter 8). With respect to level of this plastic response genetic differences were found between lines from different subsites over distances of as little as 0.5-3 m. The results confirmed the hypothesis that plants from the subsite with relatively predictable growth conditions regulate their investment in reproduction at an earlier stage of spike development, whereas plants from the shrubs (with relatively unpredictable growth conditions) adjust their maternal investment to the environmental conditions especially at a later fruiting stage.

Some general aspects (e.g. constraints) of variation in life-history characteristics of *P. major* are discussed in relation to environmental variability (chapter 9). It is concluded that levels of phenotypic plasticity varied markedly between the characteristics investigated and generally depended on the range of environments or environmental factors selected. Moreover, it is suggested that the observed genetic variation in several life-history characteristics between populations of this species were based mainly on genetic differences in plastic responses instead of genetic differences that were expressed independently of the environment. Data on both selection as well as on gene flow are needed to understand the relationship between genetic differentiation and small-scale environmental variability. Several problems of estimating gene flow within the beach-plain population are discussed. Finally, some implications of the present results on variation in life-history characteristics are discussed in relation to ecological research on the level of whole ecosystems and to applied ecology.

SAMENVATTING

Het doel van het onderzoek dat wordt beschreven in dit proefschrift, is de relatie aan te geven tussen variatie in kenmerken van de levenscyclus van een graslandsoort en variatie in eigenschappen van het milieu. Kennis ten aanzien van deze relatie is essentieel om te kunnen begrijpen hoe organismen zich aanpassen aan hun habitat. In dit opzicht is het van belang te onderzoeken of variatie in kenmerken van de levenscyclus tussen en binnen populaties is gebaseerd op genetische differentiatie of geïnduceerd wordt door het milieu (fenotypische plasticiteit). Tevens dient onderzocht te worden of verschillen in deze kenmerken, of in de mate van fenotypische plasticiteit daarin, zouden kunnen worden verklaard door verschillen in selectieregimes. Het onderzoek werd uitgevoerd aan *Plantago major* L. (grote weegbree), omdat deze meerjarige soort voorkomt in sterk verschillende habitats, bijvoorbeeld ten aanzien van struktuur en gehaltes van nutriënten in de bodem, niveau van interspecifieke concurrentie, en de aanwezigheid van milieufaktoren welke wintersterfte veroorzaken.

In een inleidende reciproke transplantatieproef en in een kasexperiment werd aangetoond dat verschillen in groei en reproductie tussen drie populaties van *P. major* ssp. *pleiosperma* in belangrijker mate door milieufaktoren, bijvoorbeeld beschikbaarheid van nutriënten, dan door genetische differentiatie werden bepaald (hoofdstuk 2). Genetische verschillen werden onder andere gevonden in zaadgrootte, leeftijd bij eerste bloei en in niveau van fenotypische plasticiteit in patronen van allocatie van droge stof naar vegetatieve en generatieve delen van de plant. Planten afkomstig van een rivieroever hadden gemiddeld kleinere zaden, bloeiden eerder en hadden een geringere fenotypische plasticiteit in biomass-allocatie naar generatieve delen, dan planten van een kwelder.

Effecten van zaadgrootte van *P. major* op kiemingspatroon en concurrentievermogen werden onderzocht in een reciproke zaaiproef en in een kasexperiment, waarin *P. major* werd gekweekt in verschillende dichtheden van *Festuca rubra* L. (rood zwenkgras) (hoofdstuk 3). Relatief kleine zaden toonden een grotere mate van kiemrust dan grote zaden. Een effect van zaadgrootte op concurrentievermogen van zaailingen kon niet worden aangetoond. Naar aanleiding van deze resultaten wordt verondersteld dat de waargenomen verschillen in zaadgrootte kunnen worden verklaard als zijnde het resultaat van verschillende selectieregimes. Planten zullen in het onvoorspelbare milieu van de rivieroever hun fitness verhogen door vele, kleine zaden te produceren. Daarentegen, produktie van kleine zaden in het kweldermilieu kan mogelijk gepaard gaan met een "kostenpost" in fitness-termen, welke is geassocieerd met een lange periode

van kiemrust in dit milieu met sterke interspecifieke concurrentie. Het vermogen van grote zaden om te kiemen kort nadat een open plaats in de vegetatie is ontstaan, bijvoorbeeld door vertrapping door vee, is hier waarschijnlijk onderhevig aan sterke selectie.

Variatie in leeftijd en plantgrootte bij eerste bloei werd uitvoerig onderzocht in een reciproke transplantatieproef met planten van vijf verschillende populaties (hoofdstuk 4). Planten van de rivieroever, een voormalige strandvlakte en een duingrasland bloeiden, gemiddeld, een jaar eerder dan planten van een pad en een kwelder. Deze genetische verschillen kwamen qua richting overeen met selectiedifferentialen van leeftijd en plantgrootte bij eerste bloei welke werden bepaald in de reproductieve fase. In tegenstelling tot op de rivieroever, de kustvlakte en in het duingrasland, verhoogden planten op het pad hun fitness door relatief laat te bloeien bij een groot formaat. Tevens wordt gesuggereerd dat ook op de kwelder planten hun fitness verhoogden door relatief laat te bloeien, aangezien in dit laatste milieu overleving tussen kieming en eerste bloei erg gering was.

Resultaten worden bediscussieerd waaruit mogelijk blijkt dat de eerder genoemde genetische verschillen tussen populaties in patronen van biomassa-allocatie (hoofdstuk 2) eveneens verklaard kunnen worden door verschillen in selectieregimes. Planten van de strandvlakte en de kwelder (beide met een lange levenscyclus bij respectievelijk lage nutriëntenrijkdom van de bodem of sterke interspecifieke concurrentie) verhoogden bij hoger aanbod van nutriënten hun biomassa-allocatie naar de bladen aanzienlijk en versterkten daarmee hun pre-reproductieve ontwikkeling, terwijl onder deze omstandigheden planten van de rivieroever (annuelle levenscyclus) juist hun reproductieve inspanning verhoogden.

Variatie in kenmerken van de levenscyclus binnen een populatie werd gedetaileerd onderzocht op de voormalige strandvlakte. In dit gebied, dat in 1966 van de zee werd afgesloten door een dijk, werd een mozaïek-patroon onderscheiden van ruimtelijke variatie in beschikbaarheid van nutriënten en water in de bodem, en in vegetatiestruktuur (hoofdstuk 5). Selectiedruk op *P. major* werd in drie verschillende submilieus onderzocht (hoofdstuk 6). In een submilieu, bestaande uit kleine gebieden (elk met een oppervlakte van circa 1 m^2) met een hoge beschikbaarheid van nutriënten en een dichte grasrijke vegetatie, hadden planten met relatief veel grote (zowel lang als breed) bladeren een hogere zaadproductie dan andere planten. In de andere submilieus, een gebied met een geringe beschikbaarheid van nutriënten en een geringe bedekking van hogere planten en een gebied met struwelen van *Hippophae rhamnoides* L. (duindoorn), was alleen bladbreedte, en niet bladlengte gecorreleerd met relatieve fitness.

De effecten van nutriëntenvoorziening en waterverzadiging van het substraat op morfologie en levenscyclus werd in een kasproef onderzocht aan lijnen afkomstig van de drie submilieus (hoofdstuk 7). Voor de meeste eigenschappen die werden bestudeerd, werd een grote mate van fenotypische plasticiteit waargenomen, welke in grootte overeenkwam met de waargenomen fenotypische variatie in *P. major* op de strandvlakte. Echter, lijnen afkomstig van de struwelen hadden in alle behandelingen een grotere verhouding tussen bladoppervlak en plantgewicht en tevens een later bloeitijdstip dan lijnen van de beide submilieus met meer open vegetatie. Tevens werd in een reciproke transplantatieproef aangetoond dat lijnen welke afkomstig waren uit de struwelen, in de schaduwrijke omgeving van deze struwelen grotere bovengrondse afmetingen, en bijvoorbeeld bredere bladeren, hadden dan andere lijnen (hoofdstuk 6).

Uit de resultaten van deze experimenten bleek niet dat lijnen afkomstig van enig submilieu speciaal aangepast waren aan specifieke niveaus van beschikbaarheid van nutriënten en water in de bodem. Er wordt dan ook verondersteld dat *P. major*, door middel van fenotypische plasticiteit, bijvoorbeeld in de morfologie van de plant, ten aanzien van deze milieufaktoren, in alle submilieus kan voorkomen en zich kan reproduceren. Ruimtelijke variatie in vegetatiestruktuur (dat wil zeggen lichtintensiteit op de bodem) veroorzaakte echter mogelijk een opsplitsing van de populatie in allocatiepatroon, bladmorfologie en in levenscyclus van *P. major* op de strandvlakte. Deze differentiatie trad op over afstanden van 15-25 m, gedurende primaire successie in een periode van maximaal 20 jaar.

Tevens werd binnen de populatie op de strandvlakte genetische differentiatie onderzocht in specifieke patronen van fenotypische plasticiteit in componenten van de zaadproductie. Deze studie werd verricht in een kasexperiment waarbij planten werden gekweekt op twee verschillende niveaus van nutriëntenvoorziening (hoofdstuk 8). Ten aanzien van de grootte van deze plastische respons werd genetische variatie gevonden tussen lijnen afkomstig van verschillende milieus over afstanden van slechts 0.5-3 m. De resultaten bevestigden de hypothese dat planten van een submilieu met relatief voorspelbare groeiomstandigheden hun investering in reproductie in een vroeg stadium van aarontwikkeling reguleren, terwijl planten van een submilieu met relatief onvoorspelbare groeiomstandigheden hun maternale investering daaraan aanpassen juist in het latere stadium van de aarafrijping.

Enige algemene aspecten van variatie in kenmerken van de levenscyclus van *P. major* worden bediscussieerd in relatie tot milieuvariatie (hoofdstuk 9). Er wordt gekonkludeerd dat de mate van fenotypische plasticiteit aanzienlijk verschilde per onderzochte eigenschap en in het algemeen afhankelijk was van de range van geselecteerde milieus en milieufaktoren.

Bovendien wordt verondersteld dat de waargenomen genetische variatie in kenmerken van de levenscyclus tussen populaties van deze soort voornamelijk gebaseerd was op genetische verschillen in plastische respons in plaats van op genetische verschillen die tot uitdrukking komen onafhankelijk van het milieu. Om de relatie tussen genetische variatie en kleischalige milieuvariatie beter te begrijpen zijn gegevens vereist over zowel selectie als "gene flow". Problemen met betrekking tot het bepalen van de verplaatsing van pollen en zaden binnen de kustvlakte-populatie worden bediscussieerd. Tenslotte, worden enige implicaties van de resultaten van dit onderzoek aan variatie in kenmerken van de levenscyclus besproken in relatie tot oecologisch onderzoek op het niveau van oecosystemen en toegepaste oecologie.

CURRICULUM VITAE

Lambertus Arie Philip Lotz werd geboren op 18 maart 1958 te Nijmegen. In 1976 deed hij examen V.W.O. aan het Ubbo Emmius Lyceum te Stadskanaal. Vanaf dat jaar studeerde hij biologie aan de Rijksuniversiteit te Groningen. In 1981 behaalde hij de kandidaatsdiploma's B1 (algemene biologie) en B5b (algemene biologie met als tweede hoofdvak biologie/ bodemkunde). Gedurende zijn studie werd hij tweemaal aangesteld als studentassistent bij de vakgroep Plantenfysiologie. In 1984 sloot hij zijn studie cum laude af met het doctoraal examen (hoofdvak plantenoecolgie, bijvakken plantenfysiologie en fysische geografie).

Van 1984 - 1986 was hij aangesteld op een promotieplaats van BION/ZWO en gedetacheerd op het Instituut voor Oecologisch Onderzoek met standplaats Oostvoorne. Het aldaar verrichte onderzoek vormt de basis voor dit proefschrift. Aansluitend volgde een aanstelling als wetenschappelijk onderzoeker op het Centrum voor Agrobiologisch Onderzoek te Wageningen met als opdracht het vermeerderen van inzicht in gewas/onkruid-interacties ten behoeve van de ontwikkeling van strategieën van onkruidbestrijding in de geïntegreerde akkerbouw.

STELLINGEN

1. Genetische verschillen tussen populaties van *Plantago major* in kenmerken van de levenscyclus zijn in belangrijke mate te herleiden tot genetische verschillen in plastische respons.
   - Dit proefschrift

2. Successie kan een essentiële factor zijn in het, binnen enige tientallen jaren, ontstaan van mozaïek-patronen in de genetische samenstelling van populaties in graslanden.
   - Dit proefschrift

3. Het feit dat *gene flow* onder natuurlijke omstandigheden zeer moeilijk te kwantificeren is, legt momenteel de grootste beperking op ten aanzien van nieuwe ontwikkelingen in de populatiebiologie.

4. Het sinds 1985 in Nederland gevoerde uitroeiingsbeleid met betrekking tot het onkruid *Cyperus esculentus* (knolcyperus) dient te worden gecontinueerd, daar dit beleid de beste bestrijdingsstrategie impliceert. De kans op werkelijke uitroeiing in Nederland is echter miniem.
   - Naber H., Rotteveel A.J.W. (1986) Meded. Fac. Landbouwwet. RU Gent 51/2a, 355-357

5. Gezien het grote maatschappelijke belang van een succesvolle invoering van geïntegreerde landbouw verdient het sterk aanbeveling na te gaan in hoeverre onkruiden als object voor fundamenteel oecologisch onderzoek kunnen worden gebruikt.

6. De huidige kennis van processen in oecosystemen is volstrekt ontoereikend om het beleid inzake afvallozing en -verwerking in voldoende mate te ondersteunen.

7. Doedelzakspel is als de liefde: door dissonanten wordt de harmonie spannend.

Stellingen behorend bij het proefschrift van L.A.P. Lotz:
Variation in life-history characteristics between and within populations of Plantago major L.

Nijmegen, 8 december 1989